B 526875

044365

APR -1 1955

ONE COPY SENT TO L. C.

VOLUME 13 NUMBER 4

NUTRITION REVIEWS

APR 12 1955

April 1955

Some Aspects of Nutrition in Yugoslavia
by Josef Brožek and Edvin Ferber 97

Clinical Nutrition
Vitamin B_{12} Concentration in Serum of Patients with Pernicious Anemia 100
The Structure and Composition of Bone in the Bantu 101
Vitamin Supplementation and Physical Performance 102
Significance of Plasma Protein Measurements in Disease States 104
Excess Weight Gain and Fatty Liver in Viral Hepatitis 105
Malnutrition and Antibody Formation 108

Nutrition Survey
Diets of Low Income Families 109

Animal Nutrition
Growth Stimulation from Synthetic Estrogens 110

Experimental Nutrition
Vitamin B_{12} Distribution in Nature 113
Insulin and Fat Metabolism 115
Antibiotics and Nitrogen Metabolism 117
Chronic Pyridoxine Deficiency in the Rat 118
Mercaptopurine in Leukemia 120
Calcium and Phosphorus Metabolism in Growing Rats 121
The Site of Conversion of Carotene to Vitamin A 123
Metabolism of Vitamin K 125

Notes
Letter to the Editor 127
Basal Metabolism of Girls in the Great Lakes Region 127
Clinical Nutrition Briefs 128

Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK 17, N. Y.

COPYRIGHT, 1955, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass.

Assistant Editor

CHRISTINE S. WILSON, One Shattuck Street, Boston

Associate Editors

JOSEF BROŽEK, PH.D., University of Minnesota, Stadium South Tower, Minneapolis
WILLIAM H. DAUGHADAY, M.D., School of Medicine, Washington University, St. Louis
CHARLES S. DAVIDSON, M.D., Thorndike Memorial Laboratory, Boston City Hospital
JAMES S. DINNING, PH.D., Department of Biochemistry, University of Arkansas School of Medicine, Little Rock
R. W. ENGEL, PH.D., Virginia Polytechnic Institute, Department of Biochemistry and Nutrition, Blacksburg
PAUL F. FENTON, PH.D., Department of Biology, Brown University, Providence
D. M. HEGSTED, PH.D., Department of Nutrition, Harvard School of Public Health, Boston
STANLEY M. LEVENSON, M.D., Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington
OLAF MICKELSEN, PH.D., Laboratory of Biochemistry and Nutrition, National Institute of Arthritis and Metabolic Diseases, Bethesda
ROBERT E. OLSON, PH.D., M.D., Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh
ROBERT E. SHANK, M.D., School of Medicine, Washington University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
WALLACE R. AYKROYD, M.D., Food and Agriculture Organization
GEORGE R. COWGILL, PH.D., Yale University
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
IAGO GALDSTON, M.D., New York Academy of Medicine
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
L. A. MAYNARD, PH.D., Cornell University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
WARREN WEAVER, PH.D., Rockefeller Foundation
JAMES R. WILSON, M.D., American Medical Association
JOHN B. YOUMANS, M.D., Vanderbilt University

Subscriptions should be sent to The Nutrition Foundation, Inc., Chrysler Building, New York 17, N.Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Entered as second-class matter at the Post Office at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

NUTRITION REVIEWS

VOL. 13 APRIL 1955 No. 4

SOME ASPECTS OF NUTRITION IN YUGOSLAVIA

Yugoslavia was born in the wake of the First World War as the Kingdom of Serbians, Croatians and Slovenians in which there were merged several administrative units widely differing in political maturity, cultural and industrial advancement, and agricultural productivity. For the most part the agricultural yield per man was low due to primitive methods of cultivation. The typical landholdings were small and the individual fields were frequently widely scattered. The population ingested a large part of its energy requirements in the form of bread, mostly corn bread, and other cereal products. The supply of animal proteins was low. Even in those areas where the supply of food was good and the people were economically in a position to eat adequate diets, the nutritional status was frequently defective due to lack of dietary information.

The Second World War ravaged the country beyond imagination. Farming was disrupted by warfare, and the domestic animals, especially horses and cattle which provided power for tilling the land, were decimated. Food was strictly rationed in the towns while rural districts had to find their own dietary salvation during these hard years. The difficulties of food supply were not removed on cessation of hostilities. With cattle reduced to less than half the prewar number, the milk supply for children was grossly inadequate. The number of hogs decreased catastrophically and the problem of supplying the population with meat and fats became critical. In order to insure a more adequate distribution of the available food, rationing of foods which were in particularly short supply (meat, milk, bread, sugar, fat) was introduced. It was abolished in 1951. In the first difficult postwar years important help was provided by UNRRA and later by UNICEF. New problems arose after the war in association with rapid industrialization and creation of large administrative centers. Many peasants migrated to towns and to sites of new factories, with inadequate housing facilities, and feeding was taken over by large canteens, put up in haste and lacking trained dietitians.

In the course of the Second World War, Yugoslavia reconstituted itself as a federation of six republics. In all the republics the medical aspects of food and nutrition are under the supervision of the regional Institutes of Hygiene. The nutritional problems vary a good deal in different republics and even within the republics. Consequently, the Institutes in the individual regions have the responsibility and unique opportunities for examining the broad impact of variations in nutrition on health and disease and for suggesting remedial measures. The Central Institutes of Hygiene at Ljubjana, Zagreb, Sarajevo, Beograd and Skopje are charged both with the hygienic control of food processing and nutritional research. Some of these Institutes are well equipped for laboratory analyses of foods as well as for dietary and nutritional surveys. The aid of WHO in providing equipment and expert consultation should be noted.

In Zagreb, the Institute has a long tradition, under B. Rogina, of chemical analysis of foodstuffs (*cf.* the work on microdetermination of iodides, Rogina and M. Dubravčić, *Analyst* **78,** *594 (1953)*). In the partisan warfare during the Second World War, in the face of severe shortages of other food, wild plants were used extensively. This stimulated systematic interest in the possibility of dietary utilization and preparation of some of the wild plants and the analysis of their nutritive content. L. Grljić reported on the ascorbic acid and carotene content of 36 edible leafy wild plants (*Acta pharm. Jugosl.* **2,** *112 (1952)*).

Many of the wild greens proved to be much better sources of ascorbic acid than similar cultivated vegetables.

Under E. Ferber in recent years intensive field work was organized in order to provide a valid picture of food consumption in different occupational groups and in the several regions of Croatia (Problems of Hygiene (Zagreb), *p. 218 (1951)*). The average caloric intake of industrial workers examined in four cities in 1949 was 3000 calories, to which bread alone contributed a full half of the total amount. For clerks the total was 2400 calories, with bread making up 1100 calories. On the average, carbohydrates contributed 80 per cent of the daily caloric intake. While the total intake of proteins was fairly satisfactory, the amounts of animal protein in the diet were low.

Preliminary information on the diet consumed by the rural population of Croatia was obtained by a survey of 250 families studied in the spring and fall of 1952 (E. Ferber, *Higijena (Zagreb)* **6,** *3 (1954)*). The foodstuffs to be used for preparation of meals on a given day were carefully weighed. The caloric value and the quality of the diet were analyzed on the basis of available tables of food consumption. The average daily diet provided 352 g. of carbohydrates, 55 g. of fats, 47 g. of vegetable and 28 g. of animal protein. It should be noted that these are *average* figures for Croatia and should not be regarded as typical of Yugoslavia as a whole. Even in Croatia there are very large regional differences, with 26 g. of daily fat intake in the mountainous, Dinaric region around Split on the Dalmatian coast, and 98 g. in the rich, hog-raising farm land of Eastern Croatia, around Osijek. The study has indicated that the menu of rural families is frequently highly monotonous and the diet, in some regions, contains meager amounts of protective nutrients.

Dietary surveys, even when carried out most meticulously, tell only a part of the story. The core of the problem is the well-being, health and productive capacity of the people. Consequently, in 1954 the dietary surveys in Croatia have been supplemented by parallel systematic surveys of nutritional status utilizing clinical, anthropometric and laboratory procedures, with R. Buzina in charge. In the framework of epidemiologic research on heart disease and in cooperation with J. Brožek and F. Mikič, Buzina carried out in 1953 an exploratory study of blood cholesterol in three regions of Croatia, markedly differing in the dietary fat intake (*cf. Zdravstvene Novine* **7,** *27 (1954)*). Earlier Buzina (*Arch. Indust. Hyg. (Zagreb)* **3,** *439 (1952)*) examined the nutritional status of industrial apprentices. B. Kesić (*Ibid.* **1,** *128 (1950)*) reported on the development of boys during adolescence, with special attention to the salutary effects of the transfer from a village to an industrial environment and improved diet.

With its long coast on the Adriatic Sea and many islands, Yugoslavia has a potentially important source of animal protein in the "fruits of the sea." Consequently, the Institute of Oceanography and Fisheries, in Split, directed by T. Šoljan, has an important role in Yugoslav food research and food technology. M. Krvarić and her coworkers examined the nutritive value of fresh Adriatic oysters (*Acta Adriatica* **5,** *79 (1953)*), and canned and fresh sardines (*Ibid.* **4,** *291 (1950)*; **5,** *123 (1953)*). S. Čmelik (*Ibid.* **3,** *127 (1948)*) has investigated the iodine content of Adriatic algae.

At the universities there are no special departments or institutes for nutritional research. However, at Zagreb the School of Public Health, under the leadership of A. Štampar, shares in nutrition education of future public health officers. Problems of food and nutrition are included in the courses for nurses, sanitary engineers and chemists. D. Hloupek is in charge of courses in home economics, offered by trained nurses and designed to bring the principles and practices of modern dietetics into the rural areas. At the Medical School of the University of Zagreb pathologic aspects of nutrition and metabolism are studied in the

Department of Internal Medicine under Professor Hahn.

In Slovenia, from the Central Institute of Hygiene in Ljubljana, studies were reported on the hygienic aspects of meat processing, the diet of the peasant population and the nutritional status of technical personnel. S. Lunaček studied growth in children (*Sodobna Pedagogika* **2,** *323 (1951)*; **4,** *71 (1951)*).

At the Central Institute of Hygiene in Belgrade such varied problems were studied as the tryptophan content of some foods, relation of diet to the hemoglobin content of blood, and body weight of students and apprentices. In addition, the Institute of Nutrition of the Serbian Academy of Sciences, now under the direction of D. Borić, has contributed importantly to nutritional research. The first volume of studies from the Institute was published in 1952 under the editorship of S. Gelineo, known for his studies on thermoregulation. The second volume appeared in 1953. It includes, among other subjects, studies on the dietary intake and growth of rural children; diet in relation to fertility, morbidity and mortality in the region of Kosovo-Metohia; and the ideas of the people in this region concerning pellagra. A. Giaja published a detailed monographic study on the diet of the inhabitants of a typical Serbian village (*Monogr. Serbian Acad. Sci., Vol. 177 (1951)*). The clinical problems of pellagra in the region of Kosovo-Metohia were examined and portrayed photographically by Borić (*Ibid., Vol. 208 (1953)*).

The medical problems of nutrition were reviewed, with special reference to Croatia, by E. Ferber (*Chapter 1* in "How Is Our Nutrition," *Zaštita Zdravlja, Zagreb (1953)*). The number of prematurely born infants is large (7.6 per cent) and reflects, at least in part, inadequate diet during pregnancy. The figure refers to births in maternity clinics. It is perhaps twice as high among children born in the homes. Infant mortality is still distressingly high, with an average of 17 per cent, and some districts reaching the incredible figures of 26 per cent (Čakavac) and 33 per cent (Djakovo). Among the avitaminoses, rickets is frequently found in children. Calcium intake has been low, due to an inadequate consumption of milk, the principal source of this mineral. In some regions it is very low. This deficiency is aggravated by the fact that much of the bread eaten is the black bread from which the calcium cannot be readily absorbed. Low hemoglobin levels are widespread.

In several areas goiter is a serious deficiency disease (*cf.* J. Matovinović, *Bull. World Health Organization* **9,** *249 (1953)*). In Croatia about 15 per cent of school children show symptoms. In one village near Zagreb 90 per cent of the population have goiter and each tenth individual shows physical or psychologic defects. The legislation concerning compulsory sale of iodized salt (Službeni List FNRJ No. 43, *p. 536 (1953)*) is an important step in the fight of the public health workers against this disease.

An important task of the nutritionist is to collect and present effectively valid data on the nutritional status of the population and its most vulnerable segments, as a basis for administrative action, educational efforts, and economic measures. Yugoslavia has a potential for supplying all of its population with adequate food and for raising a surplus of some of the products for export. There is opportunity and need for considering the physiologic needs of the human organism for food together with purely economic criteria in agricultural planning. A crucial role in improving the quantity and especially the quality of the diet will be played by the food industry, suffering at present from lack of proper equipment and technologic skill. The imbalance between the intake of animal and vegetable proteins must be eliminated by increased production of milk, dairy products and meat.

These problems are receiving increasing attention on the part of the medical profes-

sion (*cf.* E. Ferber and R. Buzina, *Zdravstvene Novine* **6,** *83 (1953)*). Current researches on medical problems of nutrition, with particular reference to iodine-deficiency goiter, were reviewed at the Second (Yugoslav) Congress for Preventive Medicine meeting at Bled, Slovenia, on September 26–30, 1954. The other two major nutritional problems in Yugoslavia are iron-deficiency anemia, and, in the south, pellagra. The results of field investigations, carried out with the support of WHO, and of a pilot study on the effectiveness of maize enrichment will be of more than local interest. Support for the maize enrichment program was provided by the Williams-Waterman Fund.

Josef Brožek
Laboratory of Physiological Hygiene
School of Public Health
University of Minnesota
Minneapolis, Minnesota

Edvin Ferber
Division of Nutrition
Central Institute of Hygiene
Zagreb, Yugoslavia

VITAMIN B_{12} CONCENTRATION IN SERUM OF PATIENTS WITH PERNICIOUS ANEMIA

Earlier studies have indicated that patients with megaloblastic anemia responding to treatment with the vitamin have relatively low serum vitamin B_{12} concentrations and that the hematopoietically active form of the vitamin is bound by serum protein (G. I. M. Ross, *J. Clin. Path.* **5,** *250 (1952)*; W. R. Pitney, M. F. Beard, and E. J. Van Loon, *J. Biol. Chem.* **207,** *143 (1954)*; *Nutrition Reviews* **12,** *164 (1954)*). A recent publication affords information concerning the concentration of vitamin B_{12} in the serum of patients with pernicious anemia and the capacity of the serum of these patients to bind the vitamin (A. A. Lear, J. W. Harris, W. B. Castle, and E. M. Fleming, *J. Lab. Clin. Med.* **44,** *715 (1954)*).

The method of assay utilizes the effect of vitamin B_{12} in stimulating the growth of the green alga, *Euglena gracilis*. The free form was determined by the simple addition of serum to the growth medium, while the total vitamin B_{12} content represented the growth of the test organism after heating of the diluted serum to 100°C. for fifteen minutes. The difference between the total and free vitamin in serum afforded a measure of the vitamin bound by serum protein.

The serums of 20 normal young adults were found to contain an average of 532 micromicrograms per milliliter of total vitamin B_{12}. Free or unbound vitamin was present in each of these serums with concentrations ranging from 27 to 104 micromicrograms per milliliter. In comparison with the values in normal persons, none of the samples of serum obtained from 33 patients with pernicious anemia before treatment contained free vitamin B_{12}, while the total serum vitamin B_{12} was reduced in all of these samples, with an average for the group of only 39 micromicrograms per milliliter. Values for other patients with pernicious anemia after treatment indicated serum concentrations of total vitamin B_{12} similar to those of normal subjects.

Comparison was also made with serum concentrations of the vitamin in patients with other types of anemia. Twelve patients with macrocytic anemia which responded to folacin were found to have serum total vitamin B_{12} concentrations somewhat below those of normal individuals, but not nearly so reduced as in the patients with pernicious anemia. The range in this group was from 148 to 557 micromicrograms per milliliter. Forty patients with anemia due to a variety of causes were included in still another

group. Among the disorders in these patients were anemias due to iron deficiency, malnutrition, myxedema, hemolytic mechanisms and a variety of chronic diseases. Total serum vitamin B_{12} values were of wide range, varying from 115 to 1029 micromicrograms per milliliter.

Similar analyses were performed on the serums obtained from 29 patients with cirrhosis of the liver. Although the range of levels of total vitamin B_{12} was very wide, 5 of these patients were observed to have extremely high serum concentrations, exceeding 1000 micromicrograms per milliliter. None of the patients had received the vitamin as treatment. In an effort to determine if the high concentrations found were produced by compounds unrelated to vitamin B_{12}, the serum of these patients was subjected to alkalinization and heating. This treatment destroys vitamin B_{12}. It was found, however, that 48 per cent of the growth-stimulating activity persisted after such treatment. Similar treatment of serum from normal persons and from patients with pernicious anemia also failed to destroy totally the growth-promoting activity. Therefore, no explanation was achieved for the relatively high values for total serum vitamin B_{12} observed in some of the patients with cirrhosis. However, it was determined that serum from all sources tested possessed a property protecting vitamin B_{12} from alkaline degradation.

The capacity of serum from normal persons and from patients with pernicious anemia to bind vitamin B_{12} added *in vitro* was studied in other experiments. It was found that the amount of vitamin B_{12} bound by serum protein increased to a maximum for each serum sample, beyond which the excess appeared as free vitamin B_{12}. The total binding capacity of serum from the patients with pernicious anemia was quantitatively similar to that of the control normal serums. In both groups excess vitamin B_{12} was found in the unbound form after the total concentration exceeded values of from 450 to 650 micromicrograms per milliliter. These observations fail to demonstrate a defect in binding of the vitamin by proteins in serum of patients with pernicious anemia. No information is afforded in these experiments which would demonstrate that binding is a property of specific serum protein fractions. Other investigators have claimed that this property resides in certain specific components of serum. R. F. Schilling and W. P. Deiss (*Proc. Soc. Exp. Biol. Med.* **83,** *506 (1953)*) found that radioactive cobalt included in the vitamin B_{12} molecule moved with the albumin fraction in the electrophoretic separation of serum proteins. Pitney and his associates (*loc. cit.*) offer evidence that it is the alpha-globulin fraction which is most important in the binding of this vitamin by serum.

The primary importance of the observations of Lear and his associates would seem to reside in the fact that they offer further confirmation of the nature of pernicious anemia as a deficiency disease. This concept is supported by the absence of detectable free vitamin B_{12} and the low total vitamin B_{12} concentration of serum of patients with this type of anemia and by the restoration of these levels to normal with vitamin B_{12} treatment.

THE STRUCTURE AND COMPOSITION OF BONE IN THE BANTU

The diet of the Bantu peoples of South Africa has been of considerable interest to nutritionists. The pathogenesis of the severe malnutrition of children, kwashiorkor, which occurs endemically in this race, has been discussed previously (*Nutrition Reviews* **9,** *129, 225 (1951)*; **10,** *87, 261, 297 (1952)*; **12,** *5 (1954)*; **13,** *1, 67 (1955)*).

Because the Bantu diet is low in vitamin D and calcium and is high in phytate phos-

phorus, A. R. P. Walker and U. B. Arvidsson (*Metabolism* **3,** *385 (1954)*) have made a study of the chemical composition and histologic structure of bone in these individuals. The fifth rib was removed at autopsy from 44 Bantu infants, 13 children and 12 adults. For comparison the ribs from 18 European infants and 22 adults were obtained. The total ash, calcium and phosphorus were determined after removal of all soft tissues and fat. In some cases the marrow was removed, a procedure which decreased the variability of the analytic results. Three of the Bantu infants presented signs of severe rickets. The dry and defatted ribs from these infants contained 43.7 per cent bone ash as compared to 59.9 per cent in nonrachitic infants. No significant difference was noted in the ash, calcium, or phosphorus content of the ribs from nonrachitic Bantu infants and European infants. The fact that 5 of the Bantu infants were suffering from kwashiorkor did not influence the percentage composition. It is probable that a low intake of vitamin D and limited exposure of babies to sunlight explained the high incidence of rickets. The authors have found the calcium concentration of breast milk from Bantu mothers to be normal despite a limited calcium intake. The analytic results on ribs from children and adults demonstrated similar composition in the Bantu and in Europeans.

The histopathologic changes in the ribs of South African Bantu infants have been described by J. Higginson (*Metabolism* **3,** *392 (1954)*). The most common change was a nonspecific growth depression which occurred in 50 per cent of the Bantu infants. This lesion was most advanced in infants dying of kwashiorkor. In these infants the zone of endochondral ossification was reduced to about one half of its normal width. Columnar and hypertrophied cartilage cells were decreased and those cells which were present were flattened and contained pyknotic nuclei. Very little invasion of blood vessels into the calcified cartilage could be seen. The trabeculae of the shaft of the bone were decreased in number and width. In some of the cases new bone formation appeared to be occurring only at the cortex.

In 27 per cent of the ribs from infants the changes of rickets were present. The lesions were severe in 5 of these cases. The frequency of growth arrest made the recognition of rickets more difficult. No significant lesions were found in 5 Bantu adolescents and 10 adult Bantus except for increased osteoid tissue suggesting rickets in a 13 year old child.

It is apparent from these two studies that although rickets is prevalent among breast-fed Bantu infants it is uncommon in childhood and in adults. Despite the low dietary intake of vitamin D and calcium and the high intake of phytate and phosphorus (components which inhibit the absorption of calcium from the gut), the percentage ash composition of most of the ribs examined was found to be normal. The histologic studies indicate a marked failure of growth of many of the ribs from Bantu infants. Caloric and protein deficiencies are probably of greater importance than mineral deficiency in most of the infants. The relative infrequence of childhood rickets and adult osteomalacia is attributed to the formation of vitamin D within the skin due to exposure to sunlight. The notion that vitamin D is not formed in significant amounts in colored subjects is not borne out by these studies.

VITAMIN SUPPLEMENTATION AND PHYSICAL PERFORMANCE

That undernutrition and malnutrition influence human emotions and behavior is well recognized, although the exact mechanisms of the complex relationships are not

established (*Nutrition Rveiews* **12,** *237* (*1954*)). Specific nutritional deficiency diseases may be accompanied by marked aberrations in mental status. Such changes are quite apparent in the dementia of pellagra, the confusion of Wernicke's disease, and the behavior and personality changes accompanying specific amino acid deficiencies as produced experimentally in human subjects. There is no question that the correction of frank nutritional disturbances when they occur is beneficial to the behavior and to the physical performance of human beings.

The possibility that beneficial effects may be derived from supplementing with vitamins the diets of industrial workers or of soldiers, especially when their rations are adequate enough to prevent the development of clinical or subclinical deficiency states, rests on much more tenuous ground. The subject of nutrition and industrial performance of workers has been previously reviewed (*Nutrition Reviews* **8,** *312* (*1950*); **11,** *239* (*1953*)). The relation of nutrition and of vitamin supplementation to physical performance is of special interest to the armed forces, since the efficiency of any military unit depends ultimately upon the maximal performance of each soldier who may be exposed to a wide variety of environmental demands during periods when strenuous activity must be exerted. R. Ryer *et al.* (*J. Clin. Nutrition* **2,** *97*, *179* (*1954*)) have reported on studies designed to determine whether the functional capacity of the soldier exposed to a cold environment might be improved by supplements of ascorbic acid and of B-complex vitamins.

Eighty-six volunteer military personnel served as test subjects. The experiment was carried out during a ten-week period in the winter of 1953 in Wyoming at an elevation of 8300 feet above sea level. The subjects were randomly divided into two groups. Forty-two men served as a control group and each received a brown capsule containing 6 mg. of ascorbic acid four times daily. Forty-four men made up the supplemented group. Each of these subjects received an orange-colored capsule four times daily which provided in each capsule the following: thiamine (10 mg.), riboflavin (10 mg.), niacinamide (100 mg.), calcium pantothenate (80 mg.), pyridoxine (40 mg.), folacin (2.5 mg.), ascorbic acid (300 mg.), and vitamin B_{12} (4 micrograms). During the experiment the average daily temperature was 26°F., wind velocity 13 miles per hour, and wind chill 1030 calories per square meter per hour. Following a period to allow for equilibration of the subjects to the prescribed conditions of diet, physical activity, and clothing, and to the cold and the altitude, measurements of physical performance were made at weekly intervals during periods of caloric adequacy (3500 calories daily during the first six weeks) and of caloric deficit (2250 calories daily during the last three weeks), under conditions of high degrees of activity, mostly outdoors in the cold. Clothing was prescribed to provide less than the amount needed to maintain comfort during inactivity in the environment of the study.

There was no statistical difference between the two experimental groups with regard to the scores attained in the Harvard step test, the Army physical fitness test, the performance with the hand dynamometer, a standardized forced march test, and a contest march. In both supplemented and unsupplemented groups performance continued to improve at the same rate during the ten weeks of study. No detectable impairment of physical performance was apparent during twenty-two days of relative caloric restriction in either group. The supplemented group demonstrated a lesser fall in rectal temperature during exposure to cold while at rest than did the control group. The differences in magnitude of the temperature decreases in the groups were small but statistically significant.

A battery of six psychologic tests failed

to reveal any differences between the two study groups. The same was true of biochemical measurements of urinary nitrogen excretion, blood glucose, hemoglobin, creatinine excretion, and 17-ketosteroid excretion. The supplemented group showed the expected increases in blood and urine levels of ascorbic acid, and both groups excreted increased amounts of oxidized ascorbic acid during exposure to cold.

The supplemented group did demonstrate significantly greater decreases in eosinophil count during forced march, and greater decreases in body weight during the course of the experiment when compared to the control group. Changes in eosinophil count were not apparent in either group following four hours of exposure to cold. The significance and interpretation of these observations are not known.

The above studies failed to demonstrate that the addition of large doses of B-vitamins and of ascorbic acid to a ration adequate enough to maintain level of performance and to prevent clinical nutritional deficiency states in soldiers undergoing physical activity in the cold improves their physical or mental performance. Until convincing evidence is presented to the contrary, it seems reasonable to assume that maximal physical performance can be expected of industrial workers or of soldiers if they are provided with adequate diets consisting of natural foodstuffs and the specific nutrients inherently contained therein, providing account is taken of the total energy requirement of the individual under prevailing circumstances of physical exertion and of environment.

SIGNIFICANCE OF PLASMA PROTEIN MEASUREMENTS IN DISEASE STATES

The blood plasma (or serum) proteins have been repeatedly studied by a variety of methods and in many disease states. In spite of these many investigations considerable doubt still exists as to the specificity of the methods, and often an unsatisfactory correlation is found between the analytic results and the character or severity of the patient's illness. Many have considered electrophoretic analysis to be the most accurate method of measurement. In general this method has shown a reduced serum albumin concentration, particularly in protein undernutrition, the nephrotic syndrome, and cirrhosis of the liver, as well as in a variety of other disease states. In inflammatory conditions some depression of the serum albumin concentration has been found associated with a rise in the alpha and gamma globulin concentrations.

Often seemingly minor or inconsistent changes have been given undue significance when related to the nature of the particular illness. It is refreshing, therefore, to find a clear-cut analysis and correlation of the clinical findings and the electrophoretic distribution of proteins in the plasma (or serum) of patients with rheumatoid arthritis. This work was done by M. W. Ropes, G. E. Perlmann, D. Kaufman, and W. Bauer (*J. Clin. Invest.* **33,** *311 (1954)*). They found a consistent decrease in the albumin concentration and a rise in the alpha-1 and alpha-2 and gamma globulin and fibrinogen concentrations. These abnormalities were usually less marked or even corrected during spontaneous, or adrenocorticotropic hormone (ACTH)-, or cortisone-induced remissions of the disease. There appeared to be little or no correlation with the duration of the disease, but the protein abnormalities did correlate well with the clinical activity adjudged by symptoms, or with the sedimentation rate.

In addition to study of the plasma proteins these authors also examined the synovial fluid protein in rheumatoid arthritis and compared it with that of the serum and

with the proteins of traumatic effusions which were considered to represent relatively normal synovial fluids (G. E. Perlmann, M. W. Ropes, D. Kaufman, and W. Bauer, *J. Clin. Invest.* **33,** *319 (1954)*). In general, the fluids from patients with mild degrees of disease and those of short duration more closely resembled the protein pattern found in traumatic arthritis. In the presence of more severe disease, and when the effusion was of long duration, there was a wide variation in the patterns. The authors suggest that the proteins in the fluid in this disease are probably due to alterations in capillary permeability, although they point out that this has not been proven.

Another critical study of the serum proteins bears upon the relation of the electrophoretic analysis, molecular weight and colloid osmotic pressure of these with particular reference to the nephrotic syndrome and cirrhosis of the liver (S. H. Armstrong *et al., J. Clin. Invest.* **33,** *297 (1954)*). The possible discrepancies between the serum colloid osmotic pressure and the serum protein concentration have been noted in the past, particularly in nutritional edema (*Nutrition Reviews* **8,** *33 (1950)*). Others have observed difficulty in correlating serum proteins determined by the Howe method ("salting out") with electrophoretic patterns and with colloid osmotic pressure measurements. The possibility exists, as noted by Armstrong and his colleagues, that in hypoproteinemic states the serum albumin in particular (which contributes most of the colloid osmotic pressure) may be present in some abnormal form; for example, larger than normal molecular aggregates. The authors used an instrument for measuring the colloid osmotic pressure which gave reproducible results. Their studies did not reveal or exclude the postulation that proteins with unusual osmotic properties might be present in some patients with hypoproteinemia, but did point up some interesting facets of the problem.

It is not necessary here to discuss the data obtained by Armstrong and his collaborators save to mention a few of the findings and the conclusions. One patient with cirrhosis of the liver, for example, who responded with improvement to "intensive dietary therapy over a period of half a year" showed essentially no change in the serum albumin and gamma globulin concentrations during this period of time, but did show a striking rise in the serum colloid osmotic pressure. The discrepancy between these two measurements in this example is striking.

The authors believe their data on the nephrotic syndrome indicate that electrophoretically determined albumin and globulin levels measured did not correlate so well with the colloid osmotic pressure as did salting out determinations (the Howe method). This they considered might be due to "shifts in the various molecular species of protein responsible for osmotic pressure." Finally, they point out that if one is to use the colloid osmotic pressure as a basis for other studies it should be measured directly rather than calculated either from the Howe values of plasma protein or from electrophoretic analysis, particularly in hypoproteinemic states. These studies further emphasize the difficulties in the measurements of the plasma proteins in disease and the need for further critical research in methods.

EXCESS WEIGHT GAIN AND FATTY LIVER IN VIRAL HEPATITIS

The acute inflammatory disease of the liver, viral hepatitis, became one of the most important medical problems confronting military personnel during World War II. It persists with relatively high incidence among civilian populations of the United States and other countries. The course of the usual or uncomplicated case of viral

hepatitis is relatively long as compared to most other acute infections. The average period of illness and convalescence requires about six weeks, making the economic costs of this illness relatively great. Moreover, convalescence may be prolonged for much longer periods and a chronic disease of the liver, cirrhosis, may follow in certain patients. There is no specific therapy for viral hepatitis, but most observations have indicated that provision of a good diet and restriction of physical activity are important in the treatment of the disease.

The currently accepted mode of dietary treatment of patients with viral hepatitis is to assure a relatively high intake of calories and protein, with provision of sufficient fat to maintain a diet that is appetizing and readily consumed (J. R. Neefe, *Am. J. Med.* **16,** *710 (1954)*). A recent report offers evidence that this type of dietary management may lead to accumulation of fat in the liver during convalescence from viral hepatitis (A. R. Colwell, *Ann. Int. Med.* **41,** *963 (1954)*).

This study was undertaken with military personnel with viral hepatitis. Small amounts of liver were obtained from each of the patients by the procedure of punch biopsy and were examined histologically. Included in the group studied were 144 patients from whom 199 liver biopsies were obtained. Physical activity was limited in a uniform manner. The patients were confined to their beds except for bathroom privileges until laboratory tests of liver function approached normal values. Food was consumed ad libitum but each patient was encouraged to eat a diet affording a high level of intake of calories, protein and carbohydrate. Although dietary intakes were not determined for each individual, the author estimated that after the first few days of hospitalization the food consumed daily by each patient afforded from 4000 to 5000 calories, from 70 to 150 g. of protein, from 400 to 500 g. of carbohydrate, and from 150 to 200 g. of fat.

Thirty-nine patients were observed during the acute course of viral hepatitis. Liver biopsies were obtained from each patient shortly after admission to the hospital and approximately two weeks after the onset of symptoms. A second biopsy was taken four weeks later, or six weeks after the onset of illness. Each liver specimen was stained and examined histologically for evidences of fibrosis and of deposition of fat in cells. The author states that the small size of the specimen did not permit the direct histochemical demonstration of cellular fat deposits. Therefore, the vacuolation of cells was utilized as an indirect measure of the accumulation of intracellular lipid. The amount of vacuolation in each tissue section was estimated and graded. Grade 0 represented sections of liver in which there was no fat or only occasional very small droplets. At the other extreme of the scale was grade 3 in which there were large, diffusely distributed vacuoles, which caused displacement of cell nuclei. Grades 1 and 2 were intermediate between these extremes. An effort was made to afford a similar quantitative estimate of the fibrous connective tissue in the stained specimens of liver.

None of the liver biopsy specimens obtained early in the acute course of the disease (*i.e.*, two weeks after onset of symptoms) was found to contain abnormal amounts of fat. However, biopsies taken four weeks later demonstrated some degree of abnormal accumulation of fat in liver cells in 21 of the 39 patients. In most instances the vacuolation of cells was of limited degree, *i.e.*, grades 1 or 2. The average grade for the 21 patients with demonstrable intracellular fat was 1.6. Although there was a minimal degree of fibrosis in about half of the group of 39 patients at this period of illness, there was no relationship apparent between fat deposition and fibrosis.

The author sought evidence of factors which might have contributed to the occurrence of fat in the livers of these patients with viral hepatitis. A variety of factors

was considered, but it was determined that the age of the patients, the severity of symptoms, and the laboratory evidences of hepatic damage were similar in the patients with and those without fat deposition in hepatic cells. One difference did seem to be of importance, however. This was the gain in body weight from the time of admission to the hospital to the date of biopsy four weeks later. The average weight gain in the patients with histologically demonstrable liver fat was 8 pounds, a value which compared with only 3 pounds in the patients without accumulation of fat in liver cells. This observation led the author to consider the possible role of excessive intake of calories and fat in causing fat deposition in the liver during the acute course of viral hepatitis. With this consideration in mind, vitamin B_{12} was administered to 20 patients in a dosage of 30 micrograms every other day during the first week of treatment. There was no apparent lipotropic or protective effect from the vitamin B_{12} administered, since one half of the group or 10 patients exhibited evidences of fatty infiltration of the liver when biopsied four weeks later.

Similar investigations were undertaken in 89 patients who had protracted courses of viral hepatitis, *i.e.*, symptoms and manifestations of disease activity persisting for periods longer than six weeks. Liver biopsy specimens were obtained at varying intervals after the onset of illness in these patients. Thirty-six of the patients had evidences of fatty changes in the liver. When compared with the 54 patients without hepatic deposition of fat, this group was similar in terms of length and severity of illness, but again the average weight gain during hospitalization was significantly higher. The average weight gain was 16 pounds in the patients with fatty infiltration and only 6 pounds in patients without this change in the liver.

The association of intracellular accumulation of fat in the livers of patients convalescing from or with persisting hepatitis has not been described by other authors. Therefore, the observations described by Colwell (*loc. cit.*) are original and new. It is of interest that this phenomenon seemed to occur in patients who had had greatest weight gain during the period of hospitalization and treatment. The evidence afforded is adequate to demonstrate that these patients were ingesting calories much in excess of their needs during the period of hospitalization and enforced limitation of physical expenditure of energy. Although the author provides evidence indicating that the severity of the disease was not significantly different between groups of patients with and without fatty infiltration of the liver, one seemingly important factor was not considered. No information is given concerning the loss of body weight during the earliest phases of viral hepatitis in these patients. There may be marked and rapid weight loss at this time. It would be of interest to know if greatest weight gains during treatment had occurred in patients whose weight loss was most severe in earlier stages of the disease.

The observations of S. Zelman (*Arch. Int. Med.* **90,** *141 (1952)*; *Nutrition Reviews* **10,** *325 (1952)*) demonstrated the frequent association of hepatic damage and liver disease with obesity. In certain patients fatty vacuolation was found in liver biopsy specimens. However, the degree of obesity was marked, with body weight more than 50 per cent in excess of ideal weight in all patients. The patients with viral hepatitis were young men of military age and there was probably little if any significant obesity, even after the weight gains which occurred during treatment and convalescence from this disease.

W. S. Hartroft (*Anat. Record* **106,** *61 (1950)*) has shown that the deposition of fat in the liver with the production of fatty cysts precedes and conditions the occurrence of fibrosis in this organ in nutritional hepatic damage in experimental animals. No evi-

dence was given in the investigation of the patients with viral hepatitis that fatty infiltration was associated with developing fibrosis of the liver, or that the vacuolation of liver cells, in itself, might be hazardous. However, the author does recommend that dietary fat and calorie intake be moderately limited so that fat deposition in the liver may possibly be avoided. This conclusion is of interest, but should be received with caution. Until more specific therapy is available for viral hepatitis, it is likely that dietary management will remain an essential part of treatment. In this instance, as in all other uses of diet in treatment, an effort must be made to afford the benefits of appropriate and nutritious food without the excesses which lead to obesity.

MALNUTRITION AND ANTIBODY FORMATION

The relationship between nutritional deficiency and the capacity to produce antibodies has received considerable study but is still controversial. There is little doubt that in severe protein deficiency in the rat antibody production is diminished (*Nutrition Reviews* **3,** *86* (*1945*); **6,** *234* (*1948*)), but the practical significance of the observation has been debated (*Ibid.* **6,** *205* (*1948*)). The studies of A. E. Axelrod and his co-workers (Symposium on Protein Metabolism, *Nutrition Symposium Series No. 8, National Vitamin Foundation, N. Y.* (*1954*)) indicate that deficiencies of pantothenic acid, pyridoxine and folacin severely impair the ability of the rat to make antibodies whereas deficiencies of riboflavin, biotin, thiamine, vitamin A and niacin produce only a moderate impairment. Deficiencies of vitamin B_{12} and vitamin D are without effect on antibody formation. These studies were done using human erythrocytes as the antigenic stimulus. Electrophoretic studies upon the serum globulins led to the conclusion that although certain vitamins decreased the ability to form antibodies, the same animals possessed a normal content of serum protein and serum globulin. The authors speculate that either there is a difference in the metabolic pathways involved in antibody formation and the production of serum globulins, or that the vitamins somehow function in the transformation of serum globulin into antibody globulin.

Comparable studies upon human beings have been very few. Both diminished (E. G. Krebs, *J. Lab. Clin. Med.* **31,** *85* (*1946*); M. G. Wohl, J. G. Reinhold, and S. B. Rose, *Arch. Int. Med.* **83,** *402* (*1949*)) and normal (H. H. Balch, *J. Immunol.* **64,** *397* (*1950*)) immunologic responses have been found in poorly nourished patients. W. P. Havens, J. M. Shaffer, and C. J. Hopke (*Ibid.* **67,** *347* (*1951*)) suggest that there appears to be a preferential synthesis of antibody in poorly nourished patients at the expense of other components of the serum proteins. Havens, D. G. Bock, and I. Siegel (*Am.J.Med.Sci.* **228,** *251* (*1954*)) have presented another study using wounded men of the Korean War who were suffering severe inanition secondary to paraplegia, tetraplegia, or because of delayed healing of wounds. A control group of 11 healthy men of comparable age were used. Purified diphtheria toxoid was used as the antigen. The anamnestic response was measured only in Schick-negative persons.

Although the 15 wounded men had lower than normal serum albumin values (range 2.0 to 4.4 g. per cent, average 3.3) compared to the control group (average 4.3 g. per cent), there was no apparent relationship between the capacity to produce antibody and serum albumin, globulin, or total serum protein level. Also, the age of the patient, clinical condition, or degree of malnutrition did not appear to be related to the antibody

response. The mean increase in diphtheria antitoxin in the severely wounded patients was slightly greater (38 units per milliliter) than in the controls (26 units per milliliter), but the variation in response within each group was large. The only significant relationship found was a general tendency for the amount of antitoxin produced to be related to the amount of pre-Schick circulating antitoxin.

It is perhaps unfortunate that the authors do not give any indication of the degree of malnutrition in the patients as judged by weight loss and similar indications, nor any information about the nutrient intake. The decrease in serum albumin level indicates that protein and presumably caloric intakes were very limited. Adequate vitamin intakes were probably maintained, although this is not mentioned in the paper. Whether the discrepancies in studies such as these and those which have been obtained with rats are explicable by differences in species response, experimental conditions, or methods, is problematic. Perhaps the most likely explanation is that only under an extreme protein deficiency, which may seldom be met in human subjects, does the level of protein intake become the governing factor in antibody response.

DIETS OF LOW INCOME FAMILIES

Food is usually the major budget item for low income families. Since adequate nutrition is essential for health it is important that food items be selected to supply the dietary requirements for these families at the lowest possible cost. In order for dietitians and public health workers to make useful suggestions concerning food purchases they should be informed as to the nutritive value of diets consumed by low income families.

P. J. Hames and E. C. Robertson (*J. Am. Dietet. Assn.* **30,** *766 (1954)*) have reported the results of a survey of the nutritive value of diets consumed by low income families in Canada. A total of five surveys of the food habits of 375 Toronto families was made between 1946 and 1948. The yearly incomes of the families surveyed ranged from $1780 to $2150 and they spent an average of 44 per cent of their income on food. The families kept a record of all food eaten for one week. Specially trained field workers aided the mothers in keeping the food records. Most of the families were surveyed more than once so that a total of 1030 records were obtained. The average intakes of calcium, thiamine, riboflavin and ascorbic acid were calculated from food composition tables (E. G. Donelson and J. M. Leichsenring, *Ibid.* **21,** *440 (1945)*). These four nutrients were selected because previous surveys had indicated that they are likely to be the limiting nutrients in most diets. The 1948 Recommended Dietary Allowances of the Food and Nutrition Board of the National Research Council (*see Nutrition Reviews* **6,** *319 (1948)*) were used to calculate the recommended intakes of these nutrients. The intakes of each family were reported as excellent when they met the recommended intake, good (80 to 99 per cent of the recommended intake), fair (50 to 79 per cent of the recommended intake), and poor (less than 50 per cent of the recommended intake).

A large number of the families received inadequate quantities of calcium and thiamine. The intakes of these nutrients equaled the recommended intakes in only approximately one fifth of the families. In contrast, 60 per cent of the families received excellent intakes of riboflavin. Ascorbic acid intakes were inadequate in a large number of the families, with 40 per cent receiving poor or fair quantities of this vitamin.

It was pointed out that the nutritive value of the diets could be considerably improved

without additional financial expense. None of the families used dried skim milk and many spent considerable money on sweet bakery products, potato chips, and canned soups. By using the money spent on the latter three items to buy dry skim milk the calcium intakes of 99 per cent of the families would have been excellent.

Thiamine intakes would have been improved by the substitution of whole grain cereal and enriched bread for the refined cereal products. This change plus the use of dry skim milk would have permitted 85 per cent of the families to obtain excellent intakes of thiamine. Changes in meat buying habits with greater use of lean pork cuts would have further improved thiamine intakes.

The changes suggested to improve thiamine and calcium intakes would also have considerably improved riboflavin intakes.

The changes in food buying habits which were suggested to improve the intakes of thiamine and calcium entailed no additional expenditure of money. These changes would result in no significant increase in the intake of ascorbic acid. A large number of families receiving inadequate intakes of this vitamin would have to purchase additional citrus fruits to meet the requirement. This would involve some additional expense.

Perhaps the most important consideration to be derived from this report is the possibility of improving the nutritive value of the diet without increasing the food budget. Field workers advising families in food purchases can do a great deal to improve the general nutritional status of the population by giving judicious advice as to which food items will supply the important nutritional factors.

GROWTH STIMULATION FROM SYNTHETIC ESTROGENS

The synthetic estrogens stilbestrol and diethylstilbestrol when implanted into growing cattle or lambs increase growth rate and efficiency of feed utilization. This observation has been reported by a number of investigators (R. M. Jordan, *J. Animal Sci.* **9,** *383 (1950)*; C. C. O'Mary *et al.*, *Ibid.* **11,** *656 (1952)*; T. M. Means, F. N. Andrews, and W. M. Beeson, *Ibid.* **12,** *176 (1953)*). The mechanism by which the estrogen implants induce body weight increases has not been established. It is of interest that these same compounds exert a growth inhibition in rats and this is explained as due to an inhibition of pituitary growth hormone.

M. T. Clegg and H. H. Cole (*J. Animal Sci.* **13,** *108 (1954)*) have reported an investigation in which attempts were made to ascertain the mechanism of growth stimulation in ruminants by synthetic estrogens. It was established that stilbestrol implants (60 mg. per steer) resulted in increased nitrogen retention. In nitrogen balance studies it was observed that this treatment had no influence on fecal nitrogen but produced a significant decrease in the urinary nitrogen excretion, thus indicating a true nitrogen storage. Nitrogen retention was also improved by synthetic estrogen in lambs in the studies by C. K. Whitehair, W. D. Gallup, and M. C. Bell (*Ibid.* **12,** *331 (1953)*) whereas O'Mary and associates (*loc cit.*) reported no difference in nitrogen retention in treated and untreated lambs.

Clegg and Cole also conducted assays to determine the effect of stilbestrol implants on adrenocorticotropic activity, and growth hormone activity. The pituitary glands of the stilbestrol-treated animals were in all cases significantly larger than those of the controls. The adrenal gland was likewise hypertrophied and histologic measurement of the cortex indicated an increase in width due to treatment. The thyroid glands of treated animals were also enlarged, but this effect

was less consistent and less pronounced than was observed with the pituitary and adrenal. The hypertrophy in the endocrine glands was consistently more pronounced in steers than in heifers.

The amount of growth hormone in the anterior pituitary of stilbestrol-treated heifers was about twice that found in control animals, based on assays with hypophysectomized rats. On the other hand, treated steers were very little different from controls, although the amount of growth hormone in the glands of treated animals was in all cases slightly less than that found in controls.

Using regression of thymus weight in 4 day old rats, assay for adrenocorticotropic hormone indicated there was no difference between treated and control animals. Stilbestrol treatment in wether and ewe lambs (12 or 24 mg. implants) produced eosinopenic responses indicative of increased adrenal cortical activity through an increased output of adrenocorticotropic hormone from the pituitary.

The study of Clegg and Cole involved 340 treated and control animals of both sexes. Although the results indicate definite effects of stilbestrol in the ruminant, they appear to be contradictory to the estrogenic effects observed in most other animal species. Certain other observations made during these studies deserve mention. Treated steers fed in dry lot or on pasture with supplemental concentrates made greater gains than controls or than treated steers on pasture alone. Stilbestrol-treated heifers in dry lot responded less than steers and revealed no increase in gain over controls when on pasture. These results suggested that the effect in heifers was somewhat conditioned by the presence of endogenous estrogens.

Stilbestrol treatment appeared to influence the carcass quality to a small extent. The per cent of treated animals in the higher carcass grades was less than the per cent of untreated; for example, only 3 treated steers attained a carcass grade of prime whereas 45 controls were in this grade. The extent of fat infiltration between muscle fibers, known as marbling, was distinctly less in the treated animals.

The predominant signs of treatment were masculinization and mammary gland development. The teats lengthened, and in a few steers some milk was found in the udder at time of slaughter seventy days after treatment. In general, these effects, however, had largely subsided by the end of ninety days following hormone implantation. In this study no attempt was made to establish the extent to which stilbestrol implants affected estrogenic activity in the tissues of treated animals.

Recognizing the possible hazard to human beings ingesting the meat of hormone-treated steers and lambs, workers at the Purdue Agricultural Experiment Station investigated the estrogenic activity of meat from treated animals (M. Stob, F. N. Andrews, M. X. Zarrow, and W. M. Beeson, *J. Animal Sci.* **13,** *138* (*1954*)). Beef was obtained from steers slaughtered one hundred and forty days after estrogen implantation (0, 60, or 120 mg. implants of stilbestrol, and 80 mg. implants of dienestrol). Lamb muscle was secured from wethers slaughtered four, eight, ten and twelve weeks after treatment (12 or 24 mg. implants of stilbestrol). Usually the meat assayed was neck and trim consisting of 70 per cent lean and 30 per cent fat. Entire chicken carcasses (treated and control) were also assayed. Treatment in chicks consisted of a 12 mg. stilbestrol pellet implanted subcutaneously.

One part of a commercial chick ration and 5 parts of the ground test meat constituted the ration, and castrated female mice served as animals in the ten-day assay. Uterine weight response was the measure of estrogenic activity. With this assay, residual estrogen could be demonstrated in the meat of treated animals. In steers both muscle and liver contained residual activity

following implantation with 120 mg. of stilbestrol whereas in lambs activity was observed only in muscle. In chicken the amount of hormone present was reduced rapidly with time after implantation whereas with lambs this loss of activity with time was slight. These workers concluded that the amount of hormone present in beef muscle and liver did not exceed 0.01 microgram per gram of dried tissue and 0.10 microgram in the case of lambs and chickens.

F. Whiting, R. D. Clark, and C. E. Allen (*Canad. J. Agr. Sci.* **34,** *288 (1954)*) reported a study in which 12-milligram pellets of diethylstilbestrol were implanted in the neck of lambs one hundred and four to one hundred and ten days prior to slaughter. Samples of kidney fat, shoulder muscle and liver were assayed for hormone activity in female rats 21 to 23 days of age. The tissue concentrations, calculated from uterine responses in the assay animals, indicated kidney fat contained 3 to 7, shoulder muscle 0.4 to 2.8 and liver 0.2 to 4 micrograms of estrogen per 100 g. of fresh tissue. In this study the authors concluded that there was no particular advantage gained from implanting diethylstilbestrol. The approximate 10 per cent increase in body weight gains that were obtained from this treatment was all accounted for in increased tissue moisture content and an increase in offal. Moisture in round of leg for treated lambs averaged 55.0 per cent, contrasted with 50.8 per cent for untreated lambs.

W. S. Wilkinson, A. L. Pope, P. H. Phillips, and L. E. Casida (*J. Animal Sci.* **13,** *684 (1954)*) reported blood and liver composition data on treated (15 mg. implant of diethylstilbestrol per lamb) and untreated lambs. Estrogen-treated lambs had lower hematocrits and higher levels of plasma (free) cholesterol and fibrinogen than controls. No effects were noted on plasma glucose, albumin, ester cholesterol, or neutral fat. Total cholesterol in plasma was increased, but not significantly. A significantly greater amount of total liver dry matter and a significantly smaller amount of liver ester cholesterol were found in the treated lambs.

The need for improved methods for the analysis of tissue residues of estrogen in animal tissues was stressed by C. E. Swift in a recent paper (*Food Research* **19,** *402 (1954)*). Swift developed a chemical test that was applied to the tissue of a lamb, a steer and poultry. The method was intended for estimation of free diethylstilbestrol. Although no evidence of the presence of synthetic estrogen was found in treated lamb or steer, small amounts were detected in the liver and skin of treated poultry. Swift concluded that further precision in methods is necessary to rule out the possibility of residues that are not detected by present procedures of combined synthetic estrogen in treated animal tissues.

Recent observation indicates that the daily oral intake of 2.5 to 10 mg. of diethylstilbestrol in fattening steers produces body weight increases of as much as 35 per cent over controls (W. Burroughs *et al.*, *Science* **120,** *66 (1954)*). These workers observed that oral synthetic estrogen produced the weight increases without some of the undesirable effects associated with the implantation of the estrogens. For example, feeding the growth stimulants appeared not to affect carcass quality, or to induce some of the undesirable changes in sexual tissues. The growth stimulatory effect from feeding synthetic estrogen has been confirmed (H. G. Luther, *et al.*, *J. Animal Sci.* **13,** *1025 (1954)*; B. Taylor, W. H. Hale, and Burroughs, *Ibid.* **13,** *1032 (1954)*).

The practice of using stilbestrol or other synthetic estrogens as a conditioning supplement in the fattening of meat animals is viewed with caution by D. W. Snair, S. E. Jaffray, H. C. Grice, and L. I. Pugsley (*Canad. J. Biochem. Physiol.* **32,** *41 (1954)*). These workers noted that, although results obtained with experimental animals (rats) cannot be compared directly to effects that may be produced in man, male rats receiv-

ing 7.5 micrograms of stilbestrol daily exhibited serious impairment in reproductive capacity.

That synthetic estrogens increase body weight gains in fattening animals seems well established. The mechanism by which this effect is brought about is, however, not clear at present, although the study of Clegg and Cole indicates strongly that endocrine gland metabolism is involved. More data are obviously needed regarding the chemical make-up of the increase in body gain and the effect on carcass quality which, based on present evidence, seems to be somewhat reduced when estrogens are used in fattening rations.

VITAMIN B_{12} DISTRIBUTION IN NATURE

It is well established that unlike other vitamins or their precursors, vitamin B_{12} is not present in significant amounts in higher green plants or in ordinary foods of vegetable origin. Such plants lack the ability to synthesize this vitamin. This is a unique situation since animal tissues also lack this ability. The sole original sources of vitamin B_{12} in nature appear to be bacteria, a few fungi, and perhaps certain other microorganisms. As yet, vitamin B_{12} has not been produced synthetically.

It is well known that vitamin B_{12} (once called the "animal-protein factor") is widely distributed in foods of animal origin, such as meat, milk, eggs, and products derived therefrom (*Nutrition Reviews* **7,** *210* (*1949*)). Best sources of vitamin B_{12} are dried cells of a variety of bacteria and fungi. Such residues are widely used in animal feeds. Several papers giving results of assays of animal tissues, foods and feedstuffs are available, including those of H. T. Peeler and co-workers (*J. Nutrition* **43,** *49* (*1951*)), U. J. Lewis, U. D. Register, H. T. Thompson, and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **72,** *479* (*1949*)), W. J. Robbins, A. Hervey, and M. E. Stebbins (*Bull. Torrey Bot. Club* **77,** *423* (*1950*); **78,** *363* (*1951*)), and H. E. Scheid and B. S. Schweigert (*J. Nutrition* **53,** *419* (*1954*)).

Since normal animal tissues do not synthesize vitamin B_{12}, this substance in these tissues is derived from the diet (from microbial sources directly or indirectly), or from synthesis by microorganisms in the digestive tract or rumen (reviewed by T. H. Jukes and W. L. Williams in "The Vitamins," *Vol. I, p. 476, Academic Press, New York* (*1954*)).

The question of whether vitamin B_{12} is synthesized by green plants has been of fundamental interest. Even before the isolation of vitamin B_{12} a number of workers demonstrated that unidentified factor activity, later found to be vitamin B_{12}, was absent or low in plant materials (M. Rubin and H. R. Bird, *J. Biol. Chem.* **163,** *387*, *393* (*1946*); C. A. Cary and A. M. Hartman, "Yearbook of Agriculture," p. *779*, *U. S. Dept. of Agriculture* (*1947*); L. M. Zucker and T. F. Zucker, *Proc. Soc. Exp. Biol. Med.* **68,** *432* (*1948*); and others). There has been some question as to whether alfalfa and grass contain vitamin B_{12}, but recent studies with better assay procedures have demonstrated that such green plants are devoid of true vitamin B_{12} activity (E. M. Bickoff, A. L. Livingston, and N. S. Snell, *Arch. Biochem.* **28,** *242* (*1950*); R. L. Jacobs, J. F. Elam, J. H. Quisenberry, and J. R. Couch, *Poultry Sci.* **32,** *812* (*1953*)).

With this background of information the finding that various seaweeds (algae) were relatively rich sources of vitamin B_{12}, as reported by Z. G. Bánhidi and L. E. Ericson (*Acta chem. Scandinav.* **7,** *713* (*1953*)) and by Robbins *et al.* (*loc. cit.*) is of interest. The finding is important because seaweed could very conceivably become an inexpensive source of vitamin B_{12} and because

this is the highest type of plant known to contain appreciable amounts of vitamin B_{12}. As much as 1 microgram of vitamin B_{12} per gram of dried material was found, which is as rich as the best known animal products.

A recent paper by L. E. Ericson and L. Lewis (*Arkiv f. Kemi* **6,** *427 (1953)*) sheds further light on the source of vitamin B_{12} in seaweed. It was found that seaweed did not have the ability to synthesize vitamin B_{12} but that closely associated bacteria produced the vitamin with eventual concentration by seaweed.

Twenty-five different species of marine algae were investigated using *E. coli* and *L. lactis* Dorner as test organisms (cup plate method). Chromatographic and ionophoresis technics were used for separation of the various forms of vitamin B_{12}. Twenty-three species contained vitamin B_{12} in amounts varying from 0.004 to 2.8 micrograms per gram on a dry weight basis. Green types of algae were richer in vitamin B_{12} than red or brown types. It is of interest that the authors found evidence for at least four types of vitamin B_{12} in algae. One of these was a new type with properties differing from known types to which they assigned the term vitamin B_{12a}. Animal activity of this new form was not reported.

Studies were made on bacterial cultures found closely associated with seaweed. Of 34 different cultures, isolated with the use of several types of mediums, 24 partially identified cultures were able to synthesize significant amounts of vitamin B_{12} (0.005 to 0.14 micrograms per milliliter of broth). The bacteria studied were motile rods and all were facultative aerobes. They produced the same type of vitamin B_{12} factors occurring in algae, as determined by bioautographic separation; however, the proportion of pseudovitamin B_{12} to other types was higher. The bacteria cultures isolated from the algae produced a greater yield of substances with vitamin B_{12} activity when grown together than when grown separately.

It is interesting that the concentration of vitamin B_{12} in seawater itself appeared very low. No vitamin B_{12} activity could be detected in brackish water concentrated one thousand times. Attempts to adsorb the vitamin in seawater on charcoal were also unsuccessful. Thus, the amount of vitamin B_{12} found in marine algae could hardly be accounted for by direct accumulation from the seawater itself.

As further evidence for the lack of ability of the algae to synthesize vitamin B_{12}, unpublished experiments (R. Scott and L. E. Ericson) were cited by Ericson and Lewis (*loc. cit.*) in which seaweeds were grown in tanks containing radioactive cobalt under relatively sterile conditions. No indication was found that vitamin B_{12} was produced by the alga itself.

The authors concluded that the original source of vitamin B_{12} in seaweed was the bacteria living epiphytically on the algae. After production of the vitamin by the bacteria the plant is able to take up and store the factors, producing a concentration of from twenty to one hundred twenty times. Studies were made showing that vitamin B_{12} was adsorbed on the cell wall of the plant. No evidence was found that the vitamin B_{12} factors were actually needed by the algae.

Another recent study presents an improved animal assay for vitamin B_{12} activity and compares the results thereof with microbiologic activity (R. J. Lillie and co-workers, *Poultry Sci.* **33,** *686 (1954)*). In the animal assay depleted chicks, 14 days of age, were used. They were hatched from eggs obtained from hens fed a vitamin B_{12}-deficient ration. The chicks were given a diet consisting of 23 to 43 per cent of corn, 3 per cent of alfalfa meal, and 50 to 70 per cent of soybean meal, supplemented with necessary vitamins and minerals. In an assay period consisting of only fourteen days a straight line growth response was obtained with graded levels of vitamin B_{12}, from 0 to 1 microgram per 100 g. of diet. Microbiologic assays were made with *L. leichmannii* by USP procedure.

The above biologic assay was used for a

study of the distribution of vitamin B_{12} in a variety of products. By the chick assay method the best sources of vitamin B_{12} were the fermentation products used in commercial channels. These ranged from 1 to 14 micrograms per gram depending on the sample. Animal products such as fish meal, meat scraps, dried buttermilk, dried whey, dried skim milk, and fish solubles each contained less than 1 microgram per gram except for a sample of liver powder which contained 5.7 micrograms per gram. Preparations obtained from sewage sludge were especially rich in vitamin B_{12}, containing as much as 2.4 micrograms per gram in one instance. It is of interest that sewage sludge has been found to be a satisfactory source of vitamin B_{12} for the pig by H. E. Schendel and B. C. Johnson (*J. Agr. Food Chem.* **2,** *23 (1954)*). A sample of concentrated grass juice contained less than 0.1 microgram of vitamin B_{12} per gram.

When the chick assays were compared with the microbiologic assay good agreement was found in most samples of fish meal studied, as well as in the activated sewage sludge, crab meal, dried skim milk, and dried whey. Poor agreement was obtained with egg yolk which gave much higher results with the chick assay. Relatively poor agreement was also obtained with commercial vitamin B_{12} concentrates, several samples of "fish solubles," and two liver concentrates. The authors point out that other factors, such as unidentified vitamins, may have influenced the chick assay which would account for the differences obtained.

The need for further investigations on vitamin B_{12} assays is evident. In the studies of Lillie and co-workers it is obvious that factors other than vitamin B_{12} may influence the results. The influence on the assay of methionine and choline, known to spare the requirement for vitamin B_{12}, is not given. Sources of unidentified factors not containing vitamin B_{12} should be included in the ration, if possible. Another disadvantage of the suggested assay is the need for maintaining a separate colony of breeding hens and incubation facilities. Most laboratories do not have such facilities. However, in spite of these shortcomings, of which the authors were aware, it is extremely important that such comparisons between methods of assay be studied. Assays of vitamin B_{12} activity with the animal are useful as a guide for the accuracy of microbiologic assays used for routine purposes.

It is of considerable interest that only the very lowest forms of plant life are able to synthesize vitamin B_{12}. Such synthesis appears to be the original source of vitamin B_{12} regardless of where it is eventually found (in such varied sources as animal tissues, soil, pond water, plants, milk, etc.). Perhaps this is one of the principal reasons that vitamin B_{12} has such economic and nutritional importance in many farm animals and in man. Further studies on the assay and distribution of vitamin B_{12} are needed. Also needed are studies on the distribution of various forms of vitamin B_{12} and their relative microbial and animal activity.

INSULIN AND FAT METABOLISM

Human obesity is a major public health problem in its own right; moreover, the statistical association of obesity with diabetes mellitus and atherosclerosis has led many investigators to regard these disorders as being metabolically related. Thus, by extension, the whole of fat metabolism and, in particular, those factors which influence the rate at which fat accumulates and is broken down in the body have become matters of practical concern to physicians and nutritionists.

Fat synthesis and degradation are under three general forms of control: enzymatic, hormonal and nervous. It is now recognized that hormonal and nervous influences do

not initiate the reactions within cells associated with fat synthesis and fat oxidation, but regulate the rate at which such reactions proceed.

The most important hormone affecting anabolism and catabolism of fat is insulin. In diabetes mellitus and in the "diabetes" of carbohydrate starvation, fat synthesis is profoundly depressed and fat oxidation greatly accelerated. In order for glycolysis (breakdown of glucose or glycogen to pyruvate) leading to production of two-carbon fat precursors to continue, glucose must be able to enter cells readily. This it cannot do without an effective supply of insulin. It also seems likely that the process of fat synthesis from two-carbon fragments, whether derived from fat or carbohydrate, is linked to glycolysis in that the energy needed for fat synthesis must be derived from the glycolytic process.

F. X. Hausberger, S. W. Milstein, and R. J. Rutman (*J. Biol. Chem.* **208,** *431 (1954)*) have studied the influence of insulin on glucose utilization in adipose and hepatic tissues *in vitro*. They compared the relative abilities of liver and of adipose tissue to form fat by measuring the rates of conversion of radioactive glucose to fatty acids and carbon dioxide by both tissues. In normal rats, as anticipated, radioglucose was utilized for lipogenesis and was oxidized by liver and adipose tissue. By contrast, liver and adipose tissue of animals made diabetic by treatment with alloxan were incapable of converting glucose into fat. Of particular interest was the finding that insulin administration to the living diabetic animal restored the ability of the liver and adipose tissues *in vitro* to make fat, and that continuation of insulin treatment promoted high levels of fat synthesis in both tissues.

Thus, administration of insulin to the alloxan-diabetic rats not only accelerated glucose metabolism to carbon dioxide and fat but also altered the channeling of energy so that much more fat was produced. Apparently a greater percentage of the energy derived from glucose oxidation in the fatty tissues is channeled by insulin action into fat than is the case in the liver.

Hausberger and his associates calculated that under the influence of insulin the lipogenic capacity of liver *in vitro* may be enhanced to well above one hundred times the normal level, while that of adipose tissue under the same hormonal influence increases only to ten times its usual value. The same investigators point out that it is not accurate to speak of adipose tissue as being metabolically inert. Adipose tissue appears inert only if correction is not made for the large mass of triglyceride usually stored in the depots. If the large difference between lipid and protein content of liver and adipose tissue is taken into account, it can be demonstrated that adipose tissue is far more active both in oxidizing glucose and in converting glucose to fat than liver. An excess of insulin has a more marked effect on lipogenesis in both tissues than upon glucose oxidation.

In a related study, T. T. Hutchens, J. T. Van Bruggen, R. M. Cockburn, and E. S. West (*J. Biol. Chem.* **208,** *115 (1954)*) have reported the effect of fasting upon tissue lipogenesis in the rat. They compared the ability of liver, gut, carcass and skin to synthesize fat in intact adult rats fasted for one, eighteen, ninety-six and two hundred and forty hours. Acetate-1-C^{14} was used as the tracer, and incorporation as per cent of dose was reported for total fatty acid and total cholesterol fractions of the four tissues. Specific activities for these lipids and total respiratory carbon dioxide and $C^{14}O_2$ collections during the four-hour experimental periods also were determined.

Values for the one-hour fast were taken as 100 per cent and fasting responses were related to this baseline. After the eighteen-hour fast, liver fatty acids showed a drop to 17 per cent of normal in the figures for per cent incorporation. Only slightly less decrease in gut and carcass fatty acid incorporation occurred, whereas skin incorporation of the label fell to 36 per cent of normal. Differences between fatty acid incorpora-

tion in the four tissues were less apparent after ninety-six and two hundred and forty hours of fasting. Liver cholesterol incorporation also showed a considerable decrease in the first eighteen-hour period and again a great drop in the subsequent seventy-eight hours. In other tissues similar but less pronounced decreases were observed as fasting progressed.

Data on carbon dioxide collections disclosed that the total per cent incorporation of C^{14}-acetate in respiratory carbon dioxide was maintained during the fasting period.

The findings of Hutchens and co-workers indicate that oxidation of radioacetate is not impaired during fasting even though incorporation of the label into fat is greatly decreased. They regard the susceptibility of liver tissue to fasting as being consistent with the well known lability of liver glycogen stores.

In summarizing the reports of Hausberger *et al.*, and of Hutchens and associates, it may be reiterated that fat synthesis requires an intact glycolytic mechanism and, perhaps, a relative excess of two-carbon fragments. During a fast, glycolysis is curtailed because (1) carbohydrate intake ceases, (2) hormones which act to block entrance of glucose into non-nervous cells are secreted in increased amounts, and (3) insulin production is diminished. Accordingly, fat synthesis is inhibited during starvation and also in uncontrolled diabetes mellitus, presumably for the same reasons in each case.

When an excess of insulin is present in the intact animal and carbohydrate intake is not limited, glucose is readily transferred into cells and there becomes available for glycolysis. The excess portion of glucose which is not stored as glycogen or terminally oxidized in the Krebs cycle presumably is transformed via acetyl coenzyme A into fat. Since this reaction requires energy, the energy produced during glycolysis is utilized for the lipogenic process.

In addition to calling attention to the lipogenic capacity of adipose tissue, Hausberger and his associates have shown that liver and adipose tissue both synthesize fat preferentially under insulin influence. Whether such increased lipogenesis represents some direct action of insulin within the cell or merely results from an enhancing effect of insulin on cellular permeability to glucose is an unresolved question. However, the relevance of studies of this kind to the problem of obesity in man is clear. The dependence of fat synthesis upon available carbohydrate and the fact that lipogenesis can be so greatly accelerated by an excess of insulin should be important considerations when experimental weight reduction diets are planned.

ANTIBIOTICS AND NITROGEN METABOLISM

A clear-cut function of antibiotics in stimulating the growth of animals has been demonstrated in two situations. Substitution of cleanliness for antibiotic resulted in as good and in some cases better growth than could be obtained with antibiotic feed supplements in chicken experiments (*Nutrition Reviews* **10,** *270 (1952)*). Rats and chickens have been observed to make improved weight gains with antibiotic-supplemented rations if the B-vitamin supply in the ration is limited. This is indirect evidence that the antibiotic effect is due to a sparing effect on the vitamin requirements (*Ibid.* **10,** *107 (1952)*).

Some physiologic investigations have indicated that storage of energy as fat is increased when the diet of growing animals is supplemented with antibiotics (A. Black and J. W. Bratzler, *J. Nutrition* **47,** *159 (1952)*; L. K. Knoebel and Black, *Ibid.* **48,** *477 (1952)*; E. W. Hartsook and B. C. Johnson, *Ibid.* **51,** *205 (1953)*). In these investigations there was no indication that the antibiotics influenced protein requirement or storage.

R. M. Forbes (*J. Nutrition* **53,** *275 (1954)*) has reported a carefully conducted experiment designed to evaluate the influence of dietary antibiotic on nitrogen metabolism in weanling rats. A complete diet containing soybean oil meal at a level to provide 12 per cent of crude protein was used. This was supplemented with antibiotics (streptomycin and chloromycetin at levels of 100 and 20 parts per million (p.p.m.) respectively), or with methionine (0.3 per cent), or with both antibiotics and methionine. The biologic value of the protein in the basal and the three supplemented diets was determined under the conditions of equalized feed intake. The biologic value of the protein was expressed as the per cent of absorbed nitrogen of the ration that was retained by the animals for use either for maintenance or for growth (gain of nitrogen).

The effect of methionine supplementation of the basal ration was what would be expected. It stimulated growth and greatly increased nitrogen retention, which are indications of a methionine deficiency of the protein in the basal ration. In spite of increased growth, fat storage was not increased in the methionine-supplemented animals.

Antibiotic supplementation of the diet consistently resulted in slightly improved digestibility of the nitrogen in the ration. The antibiotics also significantly decreased the excretion of endogenous nitrogen. A decrease of 16.8 per cent was observed in the four trials reported. This would imply that of the nitrogen absorbed less was being used for maintaining the nitrogen-containing tissues of the body, hence more would be available for the building of new nitrogen-containing tissue (body gain of nitrogen). The net effect of the antibiotics was a slightly increased body weight gain and an improvement in the retention of feed nitrogen. This was expressed in an over-all increase in positive nitrogen balance of approximately 7.5 per cent in the four trials reported. The increased nitrogen retention was slightly greater in the absence of dietary methionine supplement than in its presence.

The observation that endogenous nitrogen excretion was lowered by antibiotic feeding prompted Forbes to investigate the basal metabolic rate. No reduction in basal metabolic rate was observed that could explain the decrease in endogenous nitrogen excretion. However, the metabolic rate data were obtained in the latter phases of the experiment when the antibiotic effect on growth was much less evident than during the first few weeks of the experiment. This is an observation that is not uncommon in antibiotic studies. In many reported experiments with chicks and pigs as well as rats there is an initial rapid response to the antibiotics, following which the growth rates tend to equalize (L. J. Pecora, *J. Nutrition* **49,** *621 (1953)*). The marked effect of the antibiotics in reducing endogenous nitrogen excretion would imply that in some way body tissue metabolism is affected directly rather than indirectly as through modification of intestinal microbial populations.

The results of Forbes are in conflict with those of Knoebel and Black (*loc. cit.*) who, through equally carefully detailed studies, were unable to detect any influence of antibiotics (streptomycin and aureomycin) on nitrogen metabolism.

CHRONIC PYRIDOXINE DEFICIENCY IN THE RAT

The major effects of pyridoxine deficiency have been established as retarded growth, microcytic anemia, skin lesions (particularly of the nose, paws and tail), increased urinary excretion of xanthurenic acid (a breakdown product of the amino acid tryp-

tophan) and alterations in the general amino acid metabolism. N. S. Olsen and W. E. Martindale (*J. Nutrition* **53,** *317 (1954)*) have re-examined pyridoxine deficiency in the rat and have contributed additional information on some of the controversial aspects of the deficiency symptoms.

Weanling male rats were fed a complete, highly purified ration for four weeks. This diet consisted of 24 per cent casein, 62 per cent dextrose, 10 per cent fat, and 4 per cent salt mixture as well as all the necessary vitamins. The initial four-week period on a complete ration served to establish the normal growth rate and blood pressure level. After such "conditioning" the animals were divided into four groups, (1) those continued on the complete ration ad libitum, (2) those receiving a commercial stock ration, (3) those made deficient by feeding the purified ration in which pyridoxal was replaced by the antimetabolite desoxypyridoxine, and (4) those receiving the complete, synthetic ration in amounts just sufficient to support body weight at the same level as the deficient ration fed to group 3. The deficient group was further subdivided after four weeks, some of the animals continuing to receive the desoxypyridoxine supplement while the remainder received simply a pyridoxine-deficient ration.

The inclusion of the antivitamin in the diet led to the appearance of deficiency symptoms in little more than one week. Weight loss, severe diarrhea, skin lesions, edematous paws and convulsions were clearly present in the deficient rats. Mortality was so high that the antivitamin was dropped from the diets of most experimental animals. The administration of pyridoxal in sufficient quantity cleared up the deficiency symptoms.

Blood pressure measurements were made at three-day intervals throughout the experiment. The various control groups (both ad libitum and restricted feeding) maintained systolic pressures very close to the starting level for all groups, 119 mm. of mercury. (The standard error of ± 1 mm. shows a remarkable consistency of blood pressure from animal to animal.) The blood pressures of the deficient rats were significantly elevated. Similarly, normal mature animals showed an elevation of blood pressure after being fed desoxypyridoxine for four weeks. The addition of pyridoxal to the ration lowered blood pressure significantly in three to four days.

The urinary excretion of xanthurenic acid (formed from the amino acid tryptophan) was increased fifteen- to twenty-fold in the pyridoxine-deficient animals. Rats receiving the complete diet in reduced amounts showed a five-fold increase in xanthurenic acid excretion. Again, administration of pyridoxal reduced xanthurenic acid excretion to normal levels. While microcytic anemia has been reported to occur in pyridoxine deficiency, Olsen and Martindale found no significant changes in red cell counts and hemoglobin values of their deficient animals. On the other hand, the total white cell counts decreased significantly in the deficient animals.

Extensive histologic studies of tissues from control and deficient animals failed to show any lesions attributable to the pyridoxine deficiency. The authors fail to state whether they used more than routine staining methods. It may be that the more refined technics of cytochemistry are necessary to reveal any structural alterations which may exist in conjunction with the known metabolic changes. The histologic part of the study did not reveal lesions of the vascular system or the kidneys which might account for the high blood pressures observed.

It is especially important to point out that at least 170 rats were employed in the four experimental and control groups. The paper shows other evidences of extensive work in the large numbers of determinations made of blood pressure, xanthurenic acid excretion, blood counts, etc.

In a companion paper (*J. Nutrition* **53,**

529 (1954)) these same authors reported on organ weights, vitamin B_6 content of liver and kidney, and oxygen consumption of liver and kidney of chronically pyridoxine-deficient and control animals. The diets and other experimental arrangements were identical with those discussed above. Organ weights, expressed per 100 g. of body weight, were increased in the deficient animals for adrenals, heart, liver and kidneys. Perhaps this effect can in part be explained on the basis that such vital organs are less affected by this specific vitamin deficiency than are tissues such as muscle which, percentage-wise, contribute more heavily to the total body weight. This offers no explanation of the differential growth under adverse conditions. The question is a part of the general problem of differential growth of organs and organisms which has long fascinated biologists.

The "vitamin B_6" content of liver and kidney was determined microbiologically using the yeast *Saccharomyces carlsbergensis* as the test organism. Growth of the yeast is in general proportional to the amount of vitamin B_6 present in the tissue preparation on which it is grown. All other essential growth factors must of course be supplied in adequate amounts. As was expected, the vitamin B_6 content of livers and kidneys from deficient animals was lower than that of control rats. These levels were quickly restored to normal (four weeks or less) when the animals were supplied with the vitamin. The term "vitamin B_6" is used in connection with a microbiologic assay of this sort because the test does not permit evaluation of the individual concentrations of the four vitamers—pyridoxine, pyridoxal, pyridoxamine and pyridoxal phosphate.

Studies of the oxygen consumption of homogenized liver and kidney gave indications of profound changes in tissue metabolism. For deficient animals the oxygen uptake of both organs was significantly diminished below that of tissue from control rats. The addition of pyridoxal directly to the respiring homogenates considerably increased the oxygen uptake of the deficient tissues but did not restore it completely to the control levels. It might be argued that the diminished oxygen uptake was due in part to the lack of a critical vitamin and in part to the restricted caloric intake of the deficient animals prior to being killed. It is unfortunate that critical controls—rats on a complete diet but with food intake restricted to the level of the deficient animals—were not used or were not reported for this part of the experiment. The authors speculate on the possible role played by the altered tissue metabolism in the causation of the elevated blood pressures observed in the pyridoxine-deficient animals. They entertain the possibility that in the absence of the vitamin certain amines, formed from the amino acids, may not be removed at a sufficiently rapid rate. Such amines are known to be capable of elevating blood pressure.

MERCAPTOPURINE IN LEUKEMIA

The use of folacin antagonists in the clinical treatment of leukemia has been widely practiced with promising results (*Nutrition Reviews* **9,** *143 (1951)*). Its success has encouraged workers in this field to study antagonists of other metabolites and growth factors for this purpose.

One recently discovered compound of interest used in the management of leukemia is 6-mercaptopurine, known to be a purine antagonist for certain bacteria. The entire subject has been reviewed by J. H. Burchenal (*Cancer Research* **14,** *615 (1954)*) who states that "it should be possible in the not too far distant future to bring the efficacy of the clinical chemotherapy of leukemia to a

level comparable to that obtained in the treatment of infectious disease." Perhaps this is an overly optimistic statement, but certainly progress is being made.

The use of 6-mercaptopurine for the treatment of leukemia and allied diseases in man was investigated by J. H. Burchenal *et al.* (*Blood* **8,** *965* (*1953*)). The antimetabolite produced good clinical and hematologic remissions in 15 out of 45 children with acute leukemia; 10 showed partial remissions. The dose used in most patients was 2.5 mg. per kilogram of body weight per day. The purine antagonist was effective in some children whose disease was resistant to folacin antagonists or to ACTH and cortisone. In contrast to folacin antagonists, 6-mercaptopurine appeared to have a beneficial effect on monocytic leukemia in children and seemed to work almost equally well on leukemias with high or low total leukocyte counts. It also has considerable value in adults with this disease (Burchenal, *Cancer Research, loc. cit.*).

Additional basic studies with 6-mercaptopurine in experimental animals have now been published. H. E. Skipper, J. R. Thomson, G. B. Elion, and G. H. Hitchings (*Cancer Research* **14,** *294* (*1954*)) studied the effect of injected 6-mercaptopurine on several mice neoplasms. The usual methods of transplanting various tumors in mice and determining the effect of the drug were followed. It was found that the purine antagonist was moderately inhibitory to Sarcoma 180 and Adenocarcinoma E 0771 but markedly inhibited Adenocarcinoma 755 at the levels tested. Also, subcutaneous growth of leukemic L 1210 was inhibited by 6-mercaptopurine, especially when the latter was given with a folacin antagonist.

A. Goldin (*J. Nat. Cancer Inst.* **14,** *1247* (*1954*)) has studied compounds which protect mice against the toxicity of high levels of 6-mercaptopurine. It was felt that knowledge of such factors would aid in the treatment of leukemia in the same manner in which citrovorum factor is used to control the toxicity of folacin antagonists in certain instances.

It was found that protection against lethal toxicity of the drug was afforded by injecting purine ribotides (including adenylic acid and guanylic acid), purine ribosides (adenosine and guanosine), and diphosphopyridine nucleotide. Pyrimidine ribotides (uridylic and cytidylic acids) were ineffective and free purines (adenine, guanine, xanthine and hypoxanthine) showed very little protective action at the levels tested. The relative inactivity of the free purines is interesting since it is known that these compounds will inhibit 6-mercaptopurine in a strain of *Lactobacillus casei* (G. B. Elion *et al.*, *J. Biol. Chem.* **202,** *647* (*1953*)).

Although these studies were made with normal mice, it will be interesting to see if any of the nucleic acid derivatives will help to control the toxicity of 6-mercaptopurine in the treatment of leukemia either in man or in experimental animals. The results with this drug look encouraging, and future studies on the effectiveness of 6-mercaptopurine in leukemia and other neoplasms will be awaited with interest. Such studies are now in progress. It should be stressed that leukemia is usually a fatal disease and there is no known permanent cure. Studies such as these reviewed allow a hopeful attitude.

CALCIUM AND PHOSPHORUS METABOLISM IN GROWING RATS

A knowledge of the behavior of dietary calcium and phosphorus in animals is of importance in attempting to establish what should be the desirable intake of these elements for optimum nutritional health. Radioactive forms of these elements have been employed in the University of Tennessee–Atomic Energy Commission Agri-

cultural Research program to learn more precisely their behavior in farm animals (S. L. Hansard, C. L. Comar, and G. K. Davis, *Am. J. Physiol.* **177,** *383 (1954)*; Hansard, Comar, and M. P. Plumlee, *J. Animal Sci.* **13,** *25 (1954)*). Out of these studies came a procedure which permitted the partition of calcium in fecal excretions into endogenous (that coming from the body) and exogenous (that coming from food). The procedure is based on integrating fecal calcium excretion data from animals dosed orally with that from similar animals dosed intraperitoneally with radioactive calcium. These workers have recently investigated the behavior of calcium and phosphorus as influenced by the level of these elements in the diet of the growing rat (Hansard and Plumlee, *J. Nutrition* **54,** *17 (1954)*).

The first study of this series was designed to determine the effect of four levels of dietary calcium (0.013, 0.3, 0.5 and 1.0 per cent) on the physiologic behavior of radiocalcium (Ca^{45}) in the rat. The four diets were identical except for calcium carbonate inclusion to attain these calcium levels. The phosphorus in the diets was maintained at the constant level of 0.4 per cent. Weanling rats (25 per group) were maintained on these diets for a conditioning period of fifty-five days. The animals on the 0.013 per cent calcium diet exhibited symptoms of low calcium rickets at this time. Following the conditioning period all animals were given a single dose of Ca^{45}. Food intake and fecal and urinary collections were made during a ninety-six-hour balance study before the rats were killed for tissue analysis.

Earlier findings were substantiated in that body stores of calcium materially affected the relative absorption of calcium from the intestinal tract and excretion of body calcium into the tract. In rats that were on the low calcium level a high percentage of the total calcium excreted came from body stores (endogenous calcium). However, the total calcium excreted from body sources increased rapidly with increasing amounts of dietary calcium fed during the conditioning period. This increase in body calcium excretion persisted at all levels of dietary calcium studied. The endogenous calcium losses per kilogram of body weight per day were 8, 23, 41 and 50 mg. respectively for animals receiving 0.013, 0.3, 0.5 and 1.0 per cent of dietary calcium. These results indicate that the animal adjusts its metabolic calcium losses to the dietary calcium available. Rats on the low calcium diet absorbed 98 per cent of the dietary calcium (true digestibility value) whereas those on the high calcium diet absorbed only 45 per cent.

From these results one might expect that calcium maintenance requirements would be greatly influenced by the nutritional status of the animal with respect to calcium. Hansard and Plumlee found actually that in the young growing rat receiving 24 mg. of calcium per day the maintenance requirement was 5 mg., whereas when it had been receiving 100 mg. of calcium daily during the conditioning period, 21 mg. were required to maintain balance. This obviously indicates that the animal's ability to adjust to the calcium maintenance requirement is quite extensive. It emphasizes again that the calcium requirement must be defined in terms of the nutritional status of the animal being maintained. This is in agreement with the observations of calcium maintenance requirements reported on adult men (D. M. Hegsted, I. Moscoso, and C. Collazos, *J. Nutrition* **46,** *181 (1952)*).

The animals receiving 0.3 to 1.0 per cent of dietary calcium all contained about the same level of tissue calcium. Bone depletion of both calcium and phosphorus was severe in the animals conditioned to an intake of 0.013 per cent calcium. The incisor and molar teeth showed less evidence of depletion than mandible or tibia. The teeth apparently hold tenaciously to calcium and phosphorus and therefore may be classed with soft tissues

for preferred deposition under calcium-depletion stress.

A second phase of the study was concerned with the influence of current dietary intake of calcium on phosphorus behavior. The two dietary calcium levels selected were 0.013 and 0.5 per cent. The phosphorus level was maintained at 0.4 per cent. After the conditioning period of fifty-five days all animals were dosed with P^{32}.

Total absorption of P^{32} averaged 73 per cent for animals in low calcium status and 85 per cent for those in adequate calcium status. Balance studies indicated that 24 per cent of the P^{32} dose was lost via urine in the low calcium group whereas only 11 per cent was lost via the urine in the adequate calcium group. Since the Ca^{45} loss in the low calcium group was low it is evident that absorbed calcium and phosphorus do not behave alike, but are apparently metabolized differently. It appears that a large part of urinary phosphorus from animals on a diet adequate in this element but low in calcium is actually that which was absorbed from the food and subsequently lost via the urine. Hansard mentions unpublished data indicating a two-fold increase in both fecal-endogenous and urinary phosphorus in rats kept on low calcium diets.

In a third experiment study was made of the effects of varying the dietary phosphorus level upon the behavior of Ca^{45} when the dietary calcium was maintained at 0.5 per cent of the diet. The phosphorus levels in the diet were 0.18, 0.30, 0.50 and 1.0 per cent. Balance studies indicated no significant differences between either percentage of calcium or of Ca^{45} excreted during the balance period. Endogenous calcium loss likewise did not appear to be affected in the rats conditioned to phosphorus intakes ranging from 0.18 per cent to 1 per cent of the diet. These results would suggest that dietary phosphorus intake influenced calcium metabolism less than dietary calcium intake influenced phosphorus metabolism. It should be pointed out, however, that the lowest phosphorus level (0.18 per cent) used in the study was not low enough to establish a phosphorus-depletion stress equal to the calcium-depletion stress attained with 0.013 per cent dietary calcium.

The significant contribution of the series of isotope studies from the University of Tennessee–Atomic Energy Commission Laboratory has been that of developing procedures for obtaining estimates of endogenous mineral metabolism and furnishing means of arriving at true digestibility values for calcium and phosphorus under varying conditions of intake. These same procedures should prove valuable in determining more precisely the manner in which other factors, such as dietary mineral source (organic or inorganic), other dietary constituents, or intestinal pH, influence the metabolism of these minerals in the animal.

THE SITE OF CONVERSION OF CAROTENE TO VITAMIN A

The earlier work in this area indicated that the liver was probably the primary organ involved in converting carotene to vitamin A. However, later work by H. J. Deuel and co-workers (*see Nutrition Reviews* **6,** *92 (1948)*; **7,** *135, 169 (1949)*) showed that the intestinal wall converted large amounts of carotene to vitamin A. Their conclusion was based on the fact that when vitamin A-depleted rats were given a large dose of a beta-carotene solution vitamin A could be detected in the gastrointestinal tract before there was any change in the liver.

The evidence for the gastrointestinal wall as the primary and perhaps only organ involved in converting carotene to vitamin A was strengthened by the observation that

parenterally administered carotene was deposited in the liver of rats. There was, however, no increase in the vitamin A content of the liver following the injection of carotene (*Nutrition Reviews*, **6,** *92 (1948)*).

R. F. Krause and H. B. Pierce (*Arch. Biochem.* **19,** *145 (1948)*; (*Nutrition Reviews* **7,** *169 (1949)*) showed that even when most of the blood vessels going to the liver were ligated, rats were able to convert orally administered carotene to vitamin A. The rate of conversion under these circumstances was the same as that in normal animals. More recently, A. Rosenberg and A. E. Sobel (*Arch. Biochem. Biophys.* **44,** *320 (1953)*; *Nutrition Reviews* **12,** *23 (1954)*) showed that vitamin A could be formed when an aqueous dispersion of carotene was incubated in an isolated intestinal loop. The rate of formation of vitamin A under these circumstances was such that it could explain the formation of vitamin A when rats were given an aqueous dispersion of carotene by stomach tube.

Recently reports have appeared which point to the possibility that the liver and perhaps other organs or tissues may play some role in the conversion of carotene to vitamin A. H. D. Eaton and co-workers (*J. Dairy Sci.* **34,** *1073 (1951)*) found that when an aqueous suspension of carotene was injected into vitamin A-depleted calves there was a slight increase in the plasma vitamin A levels. There was, however, no change in liver vitamin A. K. Kowalewski, E. Henrotin, and J. van Geertruyden (*Acta gastro-enterol. belg.* **14,** *607 (1951)*) found that when colloidal carotene was injected intravenously into dogs, there was an increase in vitamin A in both the serum and the liver within two hours. J. G. Bieri and M. O. Schultze (*Arch. Biochem. Biophys.* **34,** *280 (1951)*) observed that when solubilized carotene was injected intramuscularly in vitamin A-depleted rats, small amounts of vitamin A were found in the serum and kidneys, although none was present in the liver.

In an extension of the above work, J. G. Bieri and C. J. Pollard (*Brit. J. Nutrition* **8,** *32 (1954)*) depleted weanling male rats by placing them on a vitamin A-free diet. Signs of vitamin A deficiency appeared in male animals within fifteen to eighteen days; when female rats were used, a longer period was required to produce vitamin A depletion. Crystalline all-*trans*-beta-carotene was dissolved in an aqueous solution of Tween 40. The vitamin A was determined with the antimony trichloride technic. A number of precautions were taken to be sure that the material reacting with the above reagent was actually vitamin A.

When a single dose of 50 micrograms of carotene was given to vitamin A-depleted rats, the group receiving the carotene intravenously gained 97 g., while the animals given the same dose by stomach tube gained 93 g. When a dose of 4.0 micrograms of carotene was given intravenously to vitamin A-depleted rats, they gained 44 g., while the rats given the same dose by mouth gained 36 g. The apparent superiority of the intravenously administered carotene was not seen when alpha-tocopherol was added to the supplement (to stabilize the carotene in the gastrointestinal tract).

When the animals were more severely depleted in their vitamin A stores than in the above experiments, the rats showed no response to a small dose of 5.2 micrograms of intravenously injected carotene. They did, however, respond when a dose of 18.8 micrograms of carotene was injected.

The above studies with injected carotene solutions emphasize the fact that rats were able to utilize the vitamin A which must presumably have been formed from the carotene. There was, however, the possibility that the injected carotene entered the gastrointestinal tract with the conversion occurring in the intestinal wall. F. H. Mattson, J. W. Mehl, and H. J. Deuel (*Arch. Biochem.* **15,** *65 (1947)*) had shown earlier that parenterally administered carotene may be utilized after being secreted into the

intestine by way of the bile. To rule out the latter possibility, Bieri and Pollard ligated the bile duct in vitamin A-deficient rats. Under these circumstances, the injection of carotene in aqueous dispersion was followed by the appearance of vitamin A in serum, kidney and liver. Removal of the entire small intestine resulted in no change in the rate of conversion of injected carotene to vitamin A.

The above findings convinced Bieri and Pollard that the rat can convert carotene to vitamin A in tissues other than the small intestine. The kidney was eliminated as a possible site when nephrectomized rats were studied. Removal of 60 to 75 per cent of the liver did not hamper the rat's ability to convert carotene to vitamin A. These investigators concluded that perhaps many tissues have the ability to convert carotene to vitamin A. However, under normal conditions, orally administered carotene is converted primarily in the intestinal wall. When carotene is stabilized with alpha-tocopherol, it is more efficiently utilized when given by the oral route.

Practically all workers in this field have called attention to the fact that when carotene is given by mouth there is no carotene in the serum. The suggestion has frequently been made that the rat's intestinal wall is impermeable to carotene. Another explanation for this observation may be that the rat is very efficient in converting carotene into vitamin A, while other animals, such as cattle, are not. Support for the latter suggestion comes from the work of D. C. Church and co-workers (*J. Animal Sci.* **13,** *677 (1954)*). They observed that aqueous suspensions of carotene injected into vitamin A-depleted Hereford calves produced no change in either the plasma or liver levels of vitamin A. In contrast, sheep depleted of vitamin A stores responded to the injection of carotene with an increase in the plasma vitamin A from a preinjection level of 21.4 micrograms per cent to 34 micrograms per cent one hour after the injection, and to 47.9 micrograms per cent nine hours thereafter.

It should be noted that the above observations are contrary to some of the earlier reports, especially those of Eaton and co-workers (*loc. cit.*), and R. G. Warner and L. A. Maynard (*J. Animal Sci.* **11,** *780 (1952)*). Both of these groups reported slight increases in the plasma vitamin A levels of depleted dairy calves following an injection of an aqueous suspension of carotene. Whether there are differences in the ability of beef and dairy cattle to convert carotene to vitamin A or whether the method of solubilizing the carotene is responsible for the divergent results still has to be determined.

In conjunction with the above work, it should be recalled that the level of carotene in the plasma of the calf appears to be related to the dietary intake (P. D. Boyer *et al., J. Dairy Sci.* **25,** *433 (1942)*; *Nutrition Reviews* **7,** *135 (1949)*). On this score the calf behaves the same as man. The extensive studies of the British during the last war indicated that the plasma carotene level in man is closely related to the dietary intake (E. M. Hume and H. A. Krebs, editors, *Med. Res. Council Spec. Report Series (London), No. 264 (1949)*).

Studies such as the above may be the spark which will set off a new series of investigations on the role and metabolic behavior of carotene and vitamin A.

METABOLISM OF VITAMIN K

There have been few studies of the metabolic fate of vitamin K in the animal body. Although it is required for the formation of prothrombin, the plasma proteins have no vitamin K activity (H. Dam, J. Glavind, L. Lewis, and E. Tage-Hansen, *Skand. Arch.*

Physiol. **79,** *121 (1938)*) and hence it is not a component of prothrombin. It might be expected that it would be excreted as glucuronides or sulfates and D. A. Richert (*J. Biol. Chem.* **189,** *763 (1951)*) identified 4-hydroxy-2-methyl-1-naphthyl sulfate in the urine of rabbits given about 200 mg. of 2-methyl-1,4-naphthoquinone per kilogram of body weight. Further studies have been reported by F. C. G. Hoskin, J. W. T. Spinks, and L. B. Jaques (*Canad. J. Biochem. Physiol.* **32,** *240 (1954)*) using 2-methyl-1,4-naphthoquinone (K_3) labeled with C^{14} in the methyl position. Although the doses used are still considerably above the dietary requirements, the use of the labeled compound permits investigation at a level considerably lower than would otherwise be necessary.

Adult rats were used except in one experiment where an adult guinea pig was used. The radioactive vitamin K_3 dissolved in sesame oil was injected intramuscularly at dosages from 3.8 to 11 mg. per kilogram of body weight. The urine was collected for ten to twenty hours during which time from 20 to 40 per cent of the radioactivity was excreted. Less than 10 per cent additional activity was excreted in the following twenty hours. By chromatography upon paper two products were demonstrated, both of which were different from 2-methyl-1,4-naphthoquinone itself. Product 1 was incubated with beta-glucuronidase and then was no longer distinguishable from vitamin K_3. After purification by repeated chromatography, it was analyzed for glucuronic acid and radioactivity and was found to contain approximately 2 moles of glucuronic acid per mole of vitamin. It is thus the diglucuronide.

Product 2 was not changed by glucuronidase nor by incubation with arylsulfatase (Mylase P). Yet upon analysis it was found to contain one mole of sulfate per mole of the vitamin and thus is a monosulfate ester, probably the 2-methyl-4-hydroxy-1-naphthyl sulfate described by Richert. Both products 1 and 2 were found to yield the original vitamin upon hydrolysis. This was shown by the ultraviolet absorption spectrum of the materials and by recovery of the radioactivity in authentic vitamin K_3 added to the hydrolysates and isolated as the diacetate derivative with appropriate melting point.

A third compound called product 3 was detected at low dosage levels using high specific activity material which had been overlooked in the first experiments. It was present in relatively small amounts, did not appear to be hydrolyzed by glucuronidase and did not appear to contain glucuronic acid with a modified spot test. One test for sulfate was doubtful and another was negative. After hydrolysis most of the radioactivity was recovered with authentic vitamin K_3 after addition and recovery as in the previous tests. Hence, it is an easily hydrolyzable conjugate of vitamin K_3, probably similar to the other two products described.

The authors point out that it is not possible to decide from these studies whether the products so far identified are metabolic products participating in the physiologic role of the vitamin, detoxification products, or whether there is any difference in these two possibilities. Also, it is emphasized that the results using 2-methyl-1,4-naphthoquinone are not necessarily applicable to the natural vitamins, K_1 and K_2.

NOTES

Letter to the Editor

Dear Sir:

I was interested in reading the review article "Skin Changes in Nutritional Deficiencies" appearing in the November 1954 issue of *Nutrition Reviews* (**12,** *345 (1954)*). While admitting the similarity between the histology of follicular hyperkeratosis in man and that of the skin lesion of the essential fatty acid deficiency in the rat the reviewer goes on to state:

"It is tempting to speculate that follicular hyperkeratosis is the result of essential fatty acid deficiency. This can be proved only by careful nutritional study and such a study seems to be strongly indicated."

It is clear that the reviewer is unaware of the work done earlier to test this concept. I would draw your attention to the following two articles: (1) "The Aetiology of Phrynoderma," by C. Gopalan, *Indian Med. Gaz.* **82,** *16 (1947)*; (2) "Phrynoderma: Clinical and Biochemical Investigations," by P. S. Menon, P. G. Tulpule, and V. N. Patwardhan, *Indian J. Med. Res.* **38,** *173 (1950)*.

The results of investigations described in these two papers have provided clinical and biochemical evidence that essential fatty acid deficiency was the important etiologic factor in human follicular hyperkeratosis. If your reviewer had stated that further work was necessary he would have been more correct that he has been. I trust you will not mind my pointing out to you this error of omission on the part of your reviewer.

V. N. Patwardhan, *Director*
Nutrition Research Laboratories
Indian Council of Medical Research
Coonoor, S. India

Basal Metabolism of Girls in the Great Lakes Region

Simple goiter, due to iodine deficiency, is endemic in several areas of the world. This condition is due to the low content of iodine in the soil of these regions with a consequent deficiency of iodine in the plants grown in this soil. The use of iodized table salt has greatly reduced the incidence of endemic goiter in the United States. The Great Lakes area of the United States is an area where goiter was formerly prevalent. It was estimated that 47.2 per cent of the population of Michigan had abnormal thyroid glands in 1924. At this time iodized salt was introduced in the state and a survey in 1951 indicated that the incidence of abnormal thyroids among the school children of the state had dropped to 1.4 per cent (B. E. Brush and J. K. Altland, *J. Clin. Endocrinol. Metab.* **12,** *1380 (1952)*).

Michigan has no law requiring that salt be iodized and only approximately half the salt sold contains added iodine. These considerations suggest the possibility that the iodine intake of the population of the Great Lakes region may be low. F. Kenyon, H. J. Kelly, and I. G. Macy (*J. Am. Dietet. Assn.* **30,** *987 (1954)*) have investigated this possibility. They measured the basal metabolic rates of apparently healthy Michigan girls of ages 5 to 18. The girls were wards of a children's home and lived in groups of 8 to 10 in homes in the country. The diets were considered quite adequate by the usual standards. A total of 710 observations of basal metabolic rate were made on 67 different girls. When the results were plotted as a frequency distribution it was found that the highest percentage of the observations were in the 0 to −5 per cent range of basal metabolic rates.

These experiments do not indicate a marked reduction in basal metabolic rates of these Michigan girls. However, the large number of observations used makes the trend of probable significance. It may well indicate an inadequate iodine intake for a considerable number of the subjects. It seems important to re-emphasize the necessity for using iodized salt in the goitrogenous areas of the world.

CLINICAL NUTRITION BRIEFS

"These results indicated that considerable, although not complete, protection against dental caries in Norway rats could be accomplished through inclusion in a purified diet of the ash from a protective natural diet . . . Spectrographic analyses of the ash from the natural diet revealed qualitatively the presence of at least 10 trace elements that are thus far not considered essential nutrients for animals. Whether or not any of these, rather than possibly a more optimum ratio of known elements, is responsible for better caries protection on diets containing ash than on diets containing reagent salts remains to be determined." (*Nutrition Reviews* **13,** *29* (*1955*), Minerals and Dental Caries Prevention.)

"It was found that with an oral dose of 0.5 micrograms of vitamin B_{12} the hepatic uptake was 90.5 per cent of that which occurred following the intramuscular injection. With increasing doses of vitamin B_{12} there was a rapid and progressive fall in absorption so that with doses of 50 micrograms only 3 per cent as much hepatic accumulation took place with oral as compared with intramuscular administration. Expressed in another fashion, an increase in the oral dose of vitamin B_{12} from 0.5 to 50 micrograms only results in an increase in hepatic accumulation from an estimated 0.45 to 1.5 micrograms daily." (*Nutrition Reviews* **13,** *40* (*1955*), Absorption of Vitamin B_{12}.)

"Becker and co-workers administered large amounts of calcium pantothenate to a series of 11 diabetic patients without retinopathy who had failed to demonstrate any eosinopenia following repeated corticotropin administration. In 8 of the 11 patients, pantothenate treatment was rapidly followed by a normal response to corticotropin. From these and related studies it seems clear that the defective adrenocortical function of at least some diabetic subjects without retinopathy can be attributed to a relative deficiency of pantothenate." (*Nutrition Reviews* **13,** *74* (*1955*), Vitamin Deficiencies in Patients with Diabetic Retinopathy.)

STATEMENT REQUIRED BY THE ACT OF AUGUST 24, 1912, AS AMENDED BY THE ACTS OF MARCH 3, 1933, AND JULY 2, 1946 (Title 39, United States Code, Section 233) SHOWING THE OWNERSHIP, MANAGEMENT, AND CIRCULATION OF NUTRITION REVIEWS, published monthly at Baltimore, Maryland for October 1, 1954.

1. The names and addresses of the publisher, editor, managing editor, and business managers are: Publisher: The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y., Editor: F. J. Stare, Dept. of Nutrition, Harvard University, Boston, Mass., Managing editor: none, Business manager: H. L. Sipple, Nutrition Foundation, Chrysler Bldg., N. Y., N. Y.

2. The owner is: (If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual member, must be given.) The Nutrition Foundation, Inc., Chrysler Bldg., New York 17, N. Y. (Incorporated in New York State as a non-profit foundation).

3. The known bondholders, mortgagees, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages, or other securities are: (If there are none, so state.) none

4. Paragraphs 2 and 3 include, in cases where the stockholder or security holder appears upon the books of the company as trustee or in any other fiduciary relation, the name of the person or corporation for whom such trustee is acting; also the statements in the two paragraphs show the affiant's full knowledge and belief as to the circumstances and conditions under which stockholders and security holders who do not appear upon the books of the company as trustees, hold stock and securities in a capacity other than that of a bona fide owner.

H. L. Sipple
(Business manager)

Sworn to and subscribed before me this 16th day of February, 1955.

James Aldewereld
Notary Public, State of New York
Term Expires March 30, 1956

BOARD OF TRUSTEES

*OLIVER C. CARMICHAEL, *Chairman*
University of Alabama

*FRANKLYN BLISS SNYDER, *Vice Chairman*
Evanston, Illinois

JOSEPH F. ABBOTT, *Chairman of the Board, American Sugar Refining Co.*
PAUL S. ARMSTRONG, *General Manager, Sunkist Growers*
A. C. BENJAMIN, *Vice President, Junket Brand Foods, Chr. Hansen's Laboratory, Inc.*
*F. G. BOUDREAU, *Executive Director, Milbank Memorial Fund*
CHARLES S. BRIDGES, *President, Libby, McNeill & Libby*
R. K. BRODIE, *Vice President, The Procter & Gamble Company*
G. BUITONI, *President, Buitoni Foods Corp.*
*CASON J. CALLAWAY, *Hamilton, Georgia*
*J. EARL COKE, *Assistant Secretary of Agriculture, U. S. Dept. of Agriculture*
CARLE C. CONWAY, *Chairman, Executive Committee, Continental Can Co., Inc.*
GEORGE H. COPPERS, *President, National Biscuit Company*
E. B. COSGROVE, *Chairman of the Board, Green Giant Company*
WESLEY M. DIXON, *President, Container Corporation of America*
ROGER DRACKETT, *President, Drackett Company*
*LEE A. DUBRIDGE, *President, California Institute of Technology*
*CHARLES WESLEY DUNN, *New York, New York*
EDGAR J. FORIO, *Vice President, The Coca-Cola Company*
*CLARENCE FRANCIS, *New York, New York*
DANIEL F. GERBER, *President, Gerber Products Co.*
*RUFUS C. HARRIS, *President, Tulane University*
HENRY J. HEINZ, II, *President, H. J. Heinz Co.*
*REV. THEODORE M. HESBURGH, C.S.C., *President, Notre Dame University*
JOHN HOLMES, *President, Swift & Company*
J. M. KAPLAN, *President, Welch Grape Juice Company, Inc.*
RIDGWAY KENNEDY, JR., *President, Abbotts Dairies, Inc.*
*JAMES T. KILLIAN, *President, Massachusetts Institute of Technology*
*CHARLES GLEN KING, *Scientific Director of The Nutrition Foundation*
*GRAYSON L. KIRK, *President, Columbia University*
JAMES E. KNOX, *President, Chas. B. Knox Gelatine Co., Inc.*
R. M. LAUGHLIN, *President, Continental Baking Co.*
J. PRESTON LEVIS, *Chairman, Owens-Illinois Glass Co.*
JAMES McGOWAN, JR., *Chairman of the Board, Campbell Soup Co.*
†J. S. McLEAN, *President, Canada Packers, Limited*
HANFORD MAIN, *President, Sunshine Biscuits, Inc.*
ARMAND MAY, *Chairman, American Lecithin Co., Inc.*
OSCAR MAYER, *President, Oscar Mayer & Co.*
JOHN T. MENZIES, *President, The Crosse & Blackwell Co.*
GEORGE W. MERCK, *Chairman, Merck and Co., Inc.*
JOEL S. MITCHELL, *President, Standard Brands, Inc.*
CHARLES G. MORTIMER, *President, General Foods Corporation*
H. EARLE MUZZY, *Vice Chairman, The Quaker Oats Company*
JOHN C. NAYLOR, *Executive Vice President, Pet Milk Company*
*THOMAS PARRAN, *Dean, Graduate School of Public Health, University of Pittsburgh*
RUSSELL G. PARTRIDGE, *Representing United Fruit Company*
PHILIP W. PILLSBURY, *Chairman, Pillsbury Mills, Inc.*
ROY L. PRATT, *Chairman of the Board, California Packing Corp.*
H. L. SIPPLE, *Secretary*
L. RATZESBERGER, JR., *President, The Illinois Canning Co.*
ERNEST W. REID, *President, Corn Products Refining Co.*
R. A. ROBERTSON, *President and Managing Director, George Weston, Limited*
E. J. ROCHE, *Beech-Nut Packing Company*
*LEONARD A. SCHEELE, *Surgeon General, U. S. Public Health Service*
ROBERT B. SCHNERING, *President, Curtiss Candy Company*
WILLIAM H. SHEFFIELD, JR., *President, Flako Products Corp.*
ELBERT M. SHELTON, *Secretary, The Baker Laboratories, Inc.*
WALTER SILBERSACK, *President, American Home Foods, Inc.*
*GEORGE A. SLOAN, *President of The Nutrition Foundation*
B. E. SNYDER, *President, R. B. Davis Company*
FREDERICK W. SPECHT, *President, Armour & Company*
ALFRED N. STEELE, *President, Pepsi-Cola Company*
W. C. STOLK, *President, American Can Company*
VERNON STOUFFER, *President, The Stouffer Corporation*
*LEWIS L. STRAUSS, *New York, New York*
G. CULLEN THOMAS, *Vice President, General Mills, Inc.*
L. A. VAN BOMEL, *Chairman, National Dairy Products Corp.*
W. H. VANDERPLOEG, *President, The Kellogg Company*
L. A. WARREN, *President, Safeway Stores, Inc.*
R. D. L. WIRTH, *President, Red Star Yeast and Products Co.*
C. I. WOOD, *Executive Vice President, Thomas J. Lipton, Inc.*
HARRY W. ZINSMASTER, *President, Zinsmaster Baking Co.*

---

* Representative of the public.
† Deceased.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.

A leaflet based on articles appearing currently in NUTRITION REVIEWS and entitled "Current Research in the Science of Nutrition" is distributed by The Nutrition Foundation each month. The vocabulary of the leaflet is adapted for lay reading and the subject matter is restricted to clearly established findings of practical interest. Subscriptions should be sent to The Nutrition Foundation, Inc., Chrysler Building, New York City. Rate $1.00 per year in the United States and Canada; $1.25 in other countries.